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A COMPARISON OF THE SPATIAL MAP AND WORKING MEMORY HYPOTHESES OF HIPPOCAMPAL FUNCTION

by

(C)

Timothy W. Parker

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SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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in partial fulfilment of the requirements for the degree of

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ABSTRACT

Two experiments were performed in order to investigate differing predictions from the spatial mapping hypothesis of hippocampal function proposed by O'keefe and Nadel (1978) and the working memory hypothesis proposed more recently by Olton and his colleagues.

In Experiment One groups of rats were trained to use different strategies to locate a submerged platform in a tank of water. Group MAP used a spatial mapping strategy to locate a fixed platform, and group CUE used a guidance stategy (following a suspended cue) to locate a platform which was relocated after every trial. Following acquisition half of each group was given a single low level unilateral electrical stimulation of the dentate gyrus and then run for four trials. A large interaction was found between stimulation and group, indicating that only the MAP group was impaired following stimulation.

In Experiment Two a working memory component was added to the Experiment One task, by requiring the subjects to determine on the first daily trial, which of four potential platform locations was correct, for the spatial mapping group, or which suspended cue signalled the platform, for the guidance strategy group. Working memory was required in that subjects then had to remember the results of this first trial for the remaining daily trials, in order to subsequently find the platform. Following acquisition stimulation once again selectively impaired the group using the spatial strategy, while the guidance strategy group was unimpaired. These results have important implications for the current controversy between the spatial mapping and working memory hypothesis of hippocampal function.

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INTRODUCTION

Since the original report by Scoville and Milner (1957) of the celebrated subject H. M., a concerted effort has been made to characterize memory deficits produced by interference with hippocampal function in laboratory animals. The research engendered by the original finding of severe anterograde amnesia and partial retrograde amnesia in H. M. and others, has shown hippocampal subjects to be impaired in a bewildering variety of tasks. Consequently it is not surprising that this massive body of data has lead to a large number of different hypotheses concerning the function of the hippocampus. It was the purpose of the present series of experiments to investigate two of the major hypotheses which have been advanced concerning hippocampal function, the spatial map hypothesis, and the working memory hypothesis. To begin with, though, a brief summary of some of the earlier hypotheses of hippocampal function is provided.

EARLY HYPOTHESES OF HIPPOCAMPAL FUNCTION

One interesting point concerning the majority of earlier hypotheses to be advanced is that they tended to propose a hippocampal role which did not directly involve mediation of memory processes. The prime reason for this is that it was quickly discovered that hippocampal damage to animals did not produce impairments in simple learning tasks

comparable to those of H. M. While it appeared that H. M. was unable to acquire any new information following his surgery, numerous reports from the animal literature concluded that hippocampal subjects were unimpaired in simple classical and operant conditioning tasks. These findings made it necessary to postulate something other than a pure memory deficit to account for those impairments which had been reported in the early animal literature.

For example the response inhibition hypothesis, proposed by Douglas and Pribram (1966) suggested that hippocampal subjects were unable to inhibit their responding in a task in order to switch to a more adaptive response, when the conditions of the task changed. Findings which were cited in support of this hypothesis include impairments in a wide range of tasks which involve inhibition of some ongoing resonse in order to switch to a more appropriate response. The list of such tasks in which hippocampals were found to be impaired includes habituation (Leaton, 1965; Leaton, 1967), extinction in operant conditioning (Isaacson, Douglas, & Moore, 1961; Niki, 1962; Jarrard, Isaacson, & Wickelgren, 1964; Kimble, 1968), extinction in classical conditioning (Niki, 1965), initial stages of reversal tasks (Douglas & Pribram, 1966), spontaneous alternation (Roberts, Dember & Brodwick, 1962; Douglas & Isaacson, 1964; Douglas, Peterson & Douglas,

1973), and successive discrimination (Stein & Kimble, 1966; Jarrard & Lewis, 1967; Hostetter & Thomas, 1967). Typically hippocampal subjects tend to perseverate (Peretz, 1965; Niki, 1965; Douglas & Pribram, 1966; Brown, Kaufman, & Marco, 1969; Franchina & Brown, 1970) and are quite indistractible (Wickelgren & Isaacson, 1963; Hendrickson & Kimble, 1967). Eventually, though, it became apparent that, in its original formulation, the response inhibition hypothesis was untenable. Clearly hippocampals could switch their behavior. Subjects did not starve because they could not cease a response in order to eat or drink. However it was equally clear that for some reason hippocampals did not switch responses when such changes became necessary within the context of the task. In 1972 Douglas published an hypothesis which proposed that the hippocampals were unable to attend to the relevant environmental cues which signalled that a change in responding was necessary. According to Douglas (1972) the hippocampus played an attentional role in tuning out non-reinforced stimuli. Damage to the hippocampus would result in subjects which were unable to distinguish between reinforced and non-reinforced stimuli, hence they would not exhibit behavioural changes when previously reinforced stimuli lost their reinforcement.

A reformulation of this hypothesis was provided by Solomon and Moore (1975). Basically they agreed with

Douglas (1972). However they preferred to divide the category of non-reinforced stimuli into two sub-categories, redundant and irrelevant stimuli. Support for this hypothesis was derived from studies on two attentional phenomena, latent inhibition and blocking. These acquisition phenomena were shown to be impaired by hippocampal damage (Ackil, Mellgren, Halgren, & Frommer, 1969; Solomon & Moore, 1975; Solomon, 1977; McFarland, Kostas, & Drew, 1978; Rickert, Bennett, Lane & French, 1978).

At the time much of this work was being done a radically new hypothesis of hippocampal function was being developed. O'Keefe & Nadel (1978) published an extensive work in which they outlined a pure, but highly specialized, memory function for the hippocampus. A detailed discussion of this hypothesis, which is central to the present series of experiments, is provided below.

THE SPATIAL MAP HYPOTHESIS

In 1978 O'Keefe & Nadel published their controversial book in which they proposed that the hippocampus is the brain location in which neural representations, or maps, of those environments with which the animal was familiar, reside. In support of this theory they provide a comprehensive review of the literature, focussing primarily on the results of lesion

studies, studies which correlate hippocampal EEG with behaviour and studies which correlate single unit activity with behaviour. Before discussing in more detail the formulation and implications of their theory, it is important to point out that since a map is constructed through experience, the system which controls, or mediates, its construction must be regarded as a memory system.

To introduce their theory O'Keefe & Nadel (1978) provide an initial review and discussion of the various problem-solving strategies, or hypotheses, available to normal animals faced with achieving some spatial task. In this discussion they also provide a useful formal definition of the two major memory systems, which have been commonly proposed to mediate such strategies: taxon and place. A brief summary of the capacities each provides is given below.

The Place System

To use a place strategy, an animal must possess an internal map of the environment. This map is composed of a set of place representations which are related to each other by a set of rules which represent the distances and directions amongst these places. The spatial map may be used in the following ways:

1. It allows movement from any location to any location in the environment via any route.
2. It allows the animal to locate itself within

an environment.

3. Items present within the environment can easily be located.
4. It contains a system which signals mismatches when unexpected sensory information is present. This information may take the form of an expected item not being present or an unexpected item being present.
5. The animal can navigate within an environment in which only a small subset of the original cues is still in its original location.
6. Animals which possess a spatial map can form affective relationships with specific place representations, such that not only can places be reached or recognized, but they may also be regarded as reward or punishment sites.

O'Keefe & Nadel (1978) propose that since the spatial map system resides in the hippocampus, hippocampal animals are therefore bereft of the capacities afforded by a spatial mapping system. Instead they must rely solely on the remaining memory system, the taxon system, and the strategies mediated by this system.

The Taxon System

The main difference separating the place system from the taxon system is that the taxon system only allows egocentric spatial relationships to be used. In other words

responses must depend on the immediate relationship of cue locations or cue objects to the animal, and may not depend upon relationships between remote objects or locations in the environment. The taxon system includes two distinct strategies, which may be used either separately, or in conjunction with each other. The first taxon-mediated strategy is called orientation, in which the actual motor acts required to reach or avoid a goal are specified (ie. perform the following series of behaviours to obtain a food reward in a maze: move forward five steps, turn left, move forward eight steps, turn left again, and so on). The second taxon-mediated strategy, referred to as a guidance strategy, is one in which a specific cue or cue cluster which is proximal to the object or destination is approached (or avoided), regardless of the specific motor acts required to reach the goal.

Instead of using mapping strategies, animals relying on the taxon system generate routes, which O'Keefe and Nadel (1978) describe as lists of guidance and orientation strategies. The important point about these routes is that they must always be specified with respect to the animal, and not to some external object or locations.

Since they conclude that hippocampal animals must rely solely on the taxon system, O'Keefe and Nadel (1978) spend considerable time describing the properties of the taxon system, and then evaluating the literature dealing with

various forms of hippocampal disruption, to see how it conforms to the predictions based on these properties.

Properties of the Taxon System

O'Keefe and Nadel (1978) suggest that taxon information is stored on the basis of category inclusion. A category inclusion system stores similar items in neighbouring neural circuits. A major implication of this property is that the taxon system should be particularly subject to interference effects. Thus when the same item is associated at different times with the target item, or different items are associated with the target item at the same time, strong interference would be expected to develope.

A second property of the taxon system is that the strength of the representation of an item changes incrementally as a function of experience and of the time which elapses between successive experiences. The implication here is that taxon-using animals should be particularly subject to changes in the intertrial interval employed within a task.

Properties of the Place System

In contrast with taxon properties, O'Keefe and Nadel (1978) suggest that spatial maps are formed in an all-or-none manner during active exploration upon introduction to a novel environment. The all-or-none nature of the map implies that the strength of the representation may not be

incremented once it is formed. It may become richer (more items may be introduced later) but no strengthening of existing items may occur.

O'Keefe and Nadel (1978) describe another important property of spatial maps. Since a map contains representations of items in relation to other items or locations, the same item occurring in two places will be differently represented according to its surroundings. This means that spatial maps should be subject to little interference since each item they contain is uniquely determined by the particular context in which it appears.

Since the spatial map can not be strengthened or weakened it follows that animals using spatial maps should not be affected by variations in intertrial intervals.

It can be seen that the properties discussed above generate very different predictions of how animals will behave, depending on the memory system they are employing.

The main prediction O'Keefe and Nadel (1978) make is that loss of the hippocampus should lead to the subsequent loss of two functions dependent on a place memory system. First they suggest that normal exploration upon entry to a novel environment should be impaired in some manner. Second place learning in general should be impaired, to the extent that the task in question can not easily be solved by employing alternative taxon-mediated strategies.

LITERATURE SUPPORT FOR THE SPATIAL MAP THEORY

Lesion and Stimulation Studies.

The prime necessity in evaluating the evidence O'Keefe and Nadel (1978) provide in support of their theory is that, at the very least, it should conform to their predictions concerning the behaviour of hippocampals in predominantly spatial situations. Secondly it is necessary to show that hippocampals are still able to solve tasks by resorting to taxon-mediated hypotheses. This does not mean that performance should be equally good across all tasks considered, since use of taxon-mediated strategies probably provides quite inefficient solutions to a variety of tasks easily solved by using the place system. Thirdly, it should also be the case that the literature demonstrate that hippocampals are deficient in exploration of novel objects or places.

In general (and not too surprisingly) the evidence presented by O'Keefe and Nadel (1978) provides good support for their theory. This review will deal with each of the predictions outlined above in turn.

Support from Studies on Exploration

To begin with, they deal with the literature support for a deficiency in exploratory behavior in hippocampals. There are two types of evidence to consider under this topic; reactions to novel objects introduced into familiar environments such as home cages, and exploration of wholly

novel environments. O'Keefe and Nadel (1978) suggest that in order to initially construct, and subsequently update, a spatial map, normal orienting reactions must occur in both of these situations. The literature concerning orienting responses will be considered first. To begin with initial orienting responses, in the presence of non-directed behaviour, seem to be intact in hippocampals (Crowne & Riddell, 1969; Sanwald, Porzio, Deane, & Donovick, 1970), although their subsequent exploration of novel places or objects is deficient. Glickman, Higgins, & Isaacson (1970) have shown that hippocampal gerbils explore novel objects introduced into the home cage much less than controls. O'Keefe and Nadel (1978) also cite unpublished results of Dalland (1976) who reported similar results for hippocampal rats.

Some interesting results appear when studies investigating orientation and exploration of novel objects introduced during directed behaviour are examined. A variety of studies report that when engaged in directed behaviour, hippocampal rats are quite indistractible, and show little orienting to, or exploration of, novel objects (Hendrickson, Kimble, & Kimble, 1969; Wickelgren & Isaacson, 1963). Kim (1972) reported that although strong orienting responses were absent in hippocampals engaged in directed behaviour, there were significantly more weak orienting responses. Kim (1972) distinguishes between

'strong' and 'weak' orienting responses on the basis of the presence or absence of transitory reactions (whether the animal ceases its ongoing activity or not). A non-transitory change in behavior following initial orienting is classified as a 'strong' orienting response. The implication of these results is that subjects do not appear to be impaired in their ability to perceive and shift attention to novel stimuli, but do seem to lack a tendency to subsequently explore the novel item. In cases involving directed behavior, it may be that the attention shift usually manifested by an overt orienting response is still present but is effectively masked by an overriding goal directed behavioral response.

O'Keefe and Nadel (1978) then turn to the question of exploration of novel environments by hippocampals. They describe the pattern of exploratory behaviour in normal animals as follows:

> "Typically, the animal will remain quiet for a while, perhaps sniffing about its perimeter. Slowly it will move out and explore its surrounding, often withdrawing back into areas already explored (and hence known to be safe). Once explored an area is less likely to be visited again on a subsequent foray. In time, the animal will thoroughly explore the entire situation and will become relatively quiescent, or eat if it is hungry and there is food available. At this point we can say that the animal has completed its exploration of the novel situation. On subsequent exposures to the situation the animal might make a cursory check to ensure that nothing has changed, but its activity will be much less than it was on the first occasion." (p. 255)

The conclusion they reach on the basis of their literature review is that typically hippocampals appear hyperactive but hypoexploratory. O'Keefe and Nadel (1978) cite numerous studies which describe hippocampals as showing markedly increased activity over normal levels, when placed in novel environments such as an open field apparatus (Kimble, 1963; Eichelman, 1971). Typically, though, this increased activity consists of noticeably stereotyped patterns of behaviour in which the same area of the environment is repeatedly visited. It is primarily this finding which leads O'Keefe and Nadel conclude that this increased level of activity is not due to an increased tendency to explore. A supporting finding reported by Jackson (1967) and Clark (1970) is that hippocampals exhibit a tendency to rear less than normals while in an open field, or enclosed box.

The main explanation advanced for this increased level of activity is that hippocampals are hyper-reactive to stimuli in general. A study by Kaplan (1968) demonstrates that the level of activity exhibited by hippocampals is largely determined by the nature of the external stimuli present in the testing apparatus. Rats showed classic hyperactivity in a large well-illuminated testing box, while the same rats showed no increase in activity over normals when tested in a small darkened jiggle-box. Thus it seems that external stimuli direct the behaviour of

hippocampals such that they are characterized by an increased level of general activity, consisting of stereotypic movements, directed at random toward stimuli which are present. O'Keefe and Nadel (1978) conclude that this finding:

> "demonstrates the complete absence of anything remotely comparable to exploration in hippocampal animals, their high-level activity consisting almost entirely in (sic) repetitively stereotyped behaviours; these are best described as microstereotypies, for the form of the behaviour can be remarkably constant."(p.258)

Although O'Keefe and Nadel (1978) regard the fact that hippocampals show repetitive behaviours as support for their conclusion that exploration is impaired in these subjects, it is important to point out, in passing, that this finding may be interpreted to provide support for other hypotheses of hippocampal function which postulate a less specialized role of the hippocampus in mediating memory processes. In particular the working memory hypothesis advanced by Olton and his colleagues would also predict repetitive behavior. A more elaborate discussion of this hypothesis is provided in the introduction to Experiment Two.

Support from Studies on Place Learning

It is well beyond the scope of this review to deal exhaustively with the massive literature O'Keefe and Nadel (1978) present in support of their theory. Perhaps the best strategy to follow is to begin by looking at the way

hippocampals acquire and perform tasks which are regarded as almost exclusively spatial. According to O'Keefe and Nadel (1978) these include a variety of maze learning tasks involving quite varied apparati, and explicit spatial discriminations. While there are many other tasks which may be said to contain spatial components to some degree, it is of little use to dwell on them until the more obvious spatial tasks are considered. Most important of these is maze learning.

Spatial Maze Studies. O'Keefe and Nadel (1978) begin their discussion of the spatial maze literature by reviewing Dashiell (1930). He studied acquisition by rats of a variety of related maze tasks involving a rectangular box with removable partitions. The start box and goal box were always on opposite sides of this rectangular enclosure, The fact that rats improved on this task not only within a specific configuration, but also across different configurations suggests that instead of simply chaining S-R responses, the subjects were learning about the general direction of the goal. Further support for this interpretation comes from the finding that rats tended to selectively avoid new blind alleys which led away from the goal, and were less able to avoid blind alleys which pointed in the direction of the goal. The conclusion of this study was that the rats learned some general information about the direction of the goal which they

could apply across maze configurations. Confirmation that this was the case was supplied directly by a final aspect of the Dashiell study in which rats were seen to chose various alternative paths to reach the goal when placed in mazes which allowed several different solution routes. O'Keefe and Nadel (1978) suggest that these results are compelling evidence that rats typically rely on a place strategy in complex maze tasks, rather than simply chaining fixed S-R responses. Since rats seem to prefer a place strategy in most mazes, a direct test of the O'Keefe and Nadel hypothesis may be achieved by producing hippocampal disruption during acquisition or performance of complex maze tasks. Perhaps the most compelling evidence presented by O'Keefe and Nadel (1978) is that in their list of twenty lesion papers reviewed in this category, eighteen reported a deficit for hippocampals in complex maze learning.

One of the most interesting of the early complex maze studies with hippocampals is that by Jackson and Strong (1969). The study employed a six alley, 12 cul-de-sac Lashley III maze. The task required rats to run along each alley at right angles to the goal until it reaches an opening in the wall in the direction of the goal. The rat must then turn into the alley and run along it in the opposite direction. The most interesting aspect of the task is that two types of errors are possible. First rats could miss the initial opening in the goal direction and continue

to the end of the alley, and second, once they turned into the opening they could run along the next alley in the wrong direction. Of these two erors only the first was sensitive to an impaired sense of goal direction, and it was found that hippocampals showed many more of these errors than normals.

One type of spatial maze task which has gained immense popularity over the last several years is the radial arm maze. Although O'Keefe and Nadel (1978) do not mention this task in their chapter on spatial maze impairment, many later studies employing this task provide excellent demonstrations of spatial impairment following hippocampal disruption (Olton, Walker & Gage, 1978; Becker, Walker, Olton & O'Connell, 1978; Olton & Werz, 1978; Walker & Olton, 1979; Winocur, 1982). It has also been reported that damage to more selective regions of the hippocampus is equally effective in producing spatial maze impairments. A study by Handelmann & Olton (1981) reported an impairment following lesion of CA3 pyramidal cells by kainic acid. Earlier, Jarrard (1978) showed that selective lesions of alveus, fimbria, and intrinsic CA regions produced a similar disruption of radial maze performance. In contrast, damage to other areas outside the septo-hippocampal complex seems to have little effect on spatial maze ability (Becker, Walker, Olton, & O'Connell, 1978).

Finally a quite recent body of evidence which will be discussed in the section describing the Morris water task also provides evidence of disruption of an exclusively spatial task.

Thus the conclusion based on the literature (not only that discussed specifically by O'Keefe and Nadel but also those studies published after publication of their book) is that hippocampals do seem to be impaired on a wide range of tasks which require the subject to employ place learning strategies in order to solve them efficiently. It can be seen then that the predictions made by O'Keefe and Nadel (1978) have been confirmed in the majority of cases, indicating that the theory is of value heuristically as well as being able to account for much of the early literature.

One other valuable contribution made by O'Keefe and Nadel (1978) concerns their discussion in which they provide operational definitions of problem-solving strategies. This formalization has led to increased attention to the type of strategy typically used by intact rats on a spatial task, and to increased concern that experimental groups are homogeneous with respect to the strategy they are most likely to employ in a given task. A related cause for concern is that tasks labelled "spatial" by investigators may, in fact, allow subjects to solve them without using place strategies. For example, groups of rats

may show perfect T-maze performance, but may have employed different strategies to produce this perfect performance. In order to identify the strategy actually used by the subject it is necessary to conduct probe trials. To test for orientation strategies, one can rotate the start arm 180 degrees, while keeping the goal box in a constant location. Subjects which were previously solving the task on the basis of a "turn left at the choice point" orientation strategy will now respond incorrectly. Similarly, switching a proximal cue which signals the goal to the opposite side (i.e., switch both goal arms, but leave the reward in the same location) will reveal those subjects which were employing a guidance strategy to solve the task. A case in point which effectively illustrates the danger of not controlling for the response strategy of the subject involves a paper by Munoz and Grossman (1981). These authors studied the effects of kainic-acid induced lesions of the hippocampus on T-maze performance and concluded that spatial performance on the T-maze was unimpaired, without assessing whether, in fact, the T-maze performance was due to orientation or guidance strategies. As will be described below, the conclusion of no spatial deficit has been effectively disconfirmed by Sutherland and his associates (see footnote 1).

Thus, the use of probe trials to identify strategies, and of manipulations designed either to eliminate or

encourage specific strategies (for example, see O'Keefe and Conway, 1980), has become an essential element in current research on spatial capacities of animals.

THE MORRIS WATER TASK

It is in this regard that a relatively new spatial task may be seen to be particularly useful, since it provides several important advantages over more conventional spatial task apparati, such as T-mazes or radial arm mazes, for studies with intact and brain-damaged subjects. The task has come to be known as the Morris water task (MWT) (Morris, 1980; Morris, Garrud, & Rawlins, 1981). It requires rats to swim, from different starting locations, to a submerged platform located inside a five foot diameter white fiberglass tank. The platform is rendered invisible by filling the tank with an opaque liquid (skim milk powder dissolved in cool water). The procedure is as follows: subject rats are placed into the water facing the wall and released. The path taken by the subject is then recorded by the experimenter, who also measures the latency to find the platform (submerged 2-3 cm below the surface). Some of the advantages the task provides include:

1. It is extremely simple, intact rats require only a few trials to learn it.
2. Orientation strategies are eliminated since the

rats are started from different locations around the tank circumference, thus varying the distance the subject is required to swim on each trial and the body turns needed to reach the platform.

3. Guidance strategies are also eliminated since the rat must orient using distal cues only. The submerged platform provides no proximal cues and also eliminates the possibility that the subject may lay down an odor cue of its own to mark the platform location for future reference.
4. No satiation effects occur since the subject is not motivated by a state of deprivation.

Thus the task provides an excellent control over the strategy used by subjects since only a place strategy may be used to efficiently solve the task. Since it eliminates orientation and guidance strategies alike, it is particularly well-suited for use in studies on the effects of various types of intervention in normal hippocampal neural activity on purely spatial capacities in rats. Since its original publication, the MWT has been extensively employed to show that hippocampal lesions produce severe spatial deficits in rats. Sutherland (1982) has shown that lesions from a variety of techniques, including bilateral electrolytic lesions, bilateral kainic acid-induced lesions of CA3 and CA4, and unilateral and bilateral colchicine-

induced dentate gyrus lesions, all consistently produce impairments on the MWT. Further work (Sutherland, 1982 - personal communication) has shown that damage to virtually any component of the septo-hippocampal complex (SHC), including connected areas of the frontal cortex (Kolb, Sutherland, & Whishaw, 1982), produced reliable spatial deficits. In addition, the specificity of the spatial impairment to the SHC has been amply demonstrated since lesioning a variety of extra-SHC brain structures has been ineffective in producing deficits in the MWT. Such areas include parietal cortex (Kolb, Sutherland, & Whishaw, 1982), dorso-medial thalamus (Kolb, Pittman, Sutherland, & Whishaw, 1982), habenular complex, amygdala, and motor cortex (Sutherland, Kolb, & Whishaw, 1982) and tegmental grey, substantia nigra, and fastigial nucleus of the cerebellum (Sutherland, 1982 - personal communication). Thus the SHC specificity of the spatial impairment appears quite robust. (In addition, it is worthwhile to point out that each of the lesion techniques employed in Sutherland's major study (1982) is associated with a unique combination of extra-SHC tissue invasion and damage, so that the finding of a similar deficit in all cases implies that it is the commonality shared by the lesions, namely the damage to the hippocampus, which is producing the spatial impairment.

BRIEF REVIEW OF HIPPOCAMPAL STIMULATION

In the literature lesion studies far outnumber those employing stimulation of SHC structures. Of this smaller number of studies, the majority involve consolidation paradigms, in which stimulation is applied immediately following a learning trial (i.e., Kesner and Conner, 1974; Brunner, Rossi, Stutz, and Roth, 1970) and later retention is tested.

According to Kesner and Wilburn (1974) hippocampal stimulation temporarily disrupts normal neural activity in the hippocampus and related structures, thereby producing a reversible lesion. Such a procedure would therefore be expected to produce a memory impairment similar to that demonstrated to result from hippocampal lesions.

In general this appears to be the case (Kesner and Conner, 1974; Livesey, 1975; Zornetzer, Chronister and Ross, 1973) although there have been exceptions in which hippocampal stimulation has been reported to facilitate performance of some tasks, including lever pressing for avoidance (Erickson and Patel, 1969), and operant conditioning in the mouse (Destrade, Soumireu-Mourat, and Cardo, 1973).

The stimulation employed in the present experiments consisted of low level square wave pulses delivered to the hilus of the dentate gyrus. In Experiment One unilateral stimulation was used and during surgical implanting of the

electrodes this produced a strong contralateral evoked potential, presumably through the hippocampal commissural fibres. As will be discussed more fully below, the site chosen is based on findings by Routtenberg and his colleagues that stimulation of entorhinal cortex (Collier and Routtenberg, 1978) and of the granule cells with which the entorhinal efferents synapse via the perforant pathway (Collier, Miller, Travis, and Routtenberg, 1982) produces memory disruption. The present experiments included a replication of the stimulation procedures of the Collier et al (1982) study, since they had succesfully obtained a disruption of memory effect.

Although much lesion work has been done using the MWT, to date there has been no published work on the effects of electrical stimulation of the hippocampus on spatial capacities as assessed by the MWT. In fact the literature has been particularly scanty with respect to the effects of electrical hippocampal stimulation delivered immediately prior to performance of a retention task for any type of task. For this reason, Experiment One was designed to use the stimulation procedures of Collier et al to discover whether subjects which had learned the standard version of the MWT would be impaired following stimulation of the granule cells of the hippocampal dentate gyrus.

As mentioned above, the vast majority of stimulation studies involved stimulation immediately after learning,

rather than immediately prior to or during retention tests. This issue is discussed more fully in the introduction to Experiment Two.

EXPERIMENT ONE

This experiment was designed to provide a direct test of the O'Keefe and Nadel (1978) hypothesis that hippocampal disruption should drastically impair performance of the Morris water task learned through use of place strategies, but should have no effect on Morris water task performance which is learned exclusively through guidance strategies. Although there have been a variety of reports which have provided evidence that the ability of rats to use guidance strategies is unimpaired after hippocampal damage, typically the cue tasks and spatial tasks upon which these conclusions are based do not employ the same apparati for testing the subjects. The present experimental design and tasks allow a direct comparison of the effect of hippocampal stimulation on the employment of place versus cue strategies, since the apparatus (Morris water task) and physical stimuli used are identical for all groups. The prediction underlying this experiment, based on the spatial map hypothesis of O'Keefe and Nadel (1978), is that disruption of hippocampal neural activity by electrical stimulation should produce a spatial impairment similar to that resulting from lesioned subjects. However such stimulation should have no effect on subjects trained to

solve the Morris water task by employing a guidance strategy.

It should be pointed out that no studies have been published on the effects of hippocampal lesions on ability to use a cue strategy to solve the Morris water task. However Sutherland (1982 - personal communication) reports that while acquisition of such a strategy is slightly delayed for hippocampal rats, eventually they show performance levels equal to normals. The present experiment examined a complementary situation to the studies of Sutherland in that it dealt with the effects of stimulation of the hippocampus on performance of the Morris water task by subjects trained to use either a guidance strategy or a place strategy to solve the task.

Design

During training all subjects received identical acquisition trials. Following this each group was divided into equal subgroups for a series of performance trial days during which stimulation was given to the experimental subgroups groups only over three consecutive days. The remaining subgroups acted as controls for these days. At the end of these three stimulation days each stimulated subgroup was given probe trials with a cheesecloth tent covering the tank. Following this conditions were changed so that the cue signalling the platform for the subjects

using guidance strategies was suspended over the platform instead of being on the tank wall. All subjects were given additional acquisition training with these conditions in effect. Following this the subgroups which previously acted as controls were now stimulated on two consecutive days, with the previous experimental subgroups now acting as the controls. The last part of the experiment consisted of the initial experimental subgroups receiving two additional stimulation days.

Method

Subjects

The subjects consisted of 40 male Long-Evans hooded rats, weighing between 350 and 450 gm before implantation. All subjects were housed together in a temperature-controlled room on a 24 hour continuouslight cycle and given *ad libitum* food and water throughout the course of the experiment.

Before acquisition training, all subjects received identical surgical implants of bilateral hippocampal electrode assemblies. After a minimum of two weeks for recovery acquisition trials began. (Circumstances dictated that 12 subjects received up to 4 weeks of recovery, however these subjects were divided as evenly as possible among the groups.) Prior to training, subjects were randomly allocated to two groups of twenty animals, referred to as a MAP group and a CUE group. During training

one subject from the MAP group lost its head cap and was discarded.

Surgical Procedures

Each subject was anaesthetized with sodium pentobarbital and fixed in a stereotaxic apparatus, with the incisor bar set at -3.3 mm. A mid-saggital incision was made along the scalp and the skull was bared by scraping away the periosteum. Bilateral holes were drilled using the following coordinates with respect to bregma: AP, -3.5 mm, L, 2.0 mm. An electrode assembly was then implanted in each hippocampus and fixed in place using dental cement.

In addition to the electrode holes, three other holes were drilled in the skull prior to implanting the assembly. A reference screw electrode was placed approximately four to five mm anterior to bregma, betwen the subject's eyes, and two screws which anchored the dental cement cap to the skull were placed in holes caudal to the electrodes.

Actual placement of the electrode assemblies employed the technique of recording EEG activity from the descending electrodes, described by Collier et al (1982). The molecular layer of the dentate gyrus of the hippocampus produces a very distinctive and characteristic bursting pattern when invaded by a microelectrode, hence it is relatively easy to achieve very accurate placement of electrodes in this region, by slowly lowering the electrode until this pattern appears in the EEG record.

When both assemblies had been implanted, the placements were tested by stimulating one side with a pulse of up to 12 volts at one to five Herz, and recording the evoked potential in the contralateral homologous site.

Finally the assemblies were fixed in place and the scalp wound sutured and painted with Neosporin antibiotic ointment. The subject was then removed from the stereotaxic instrument and replaced in the home cage for recovery.

Preparation of Electrode Assemblies

The individual electrodes which were employed in the assemblies were constructed using a technique outlined by Vanderwolf & Cooley (1978). The insulation was scraped from the top of a length of fine Teflon-coated stainless steel wire (0.0092 mm with Trimethyl insulation, commercially available from Johnson Mathey Metal Ltd.) which was then soldered to the short end of a female Amphenol microconnector, using phosphoric acid as a flux. Finally a short length (3-4 mm) of black polyvinyl tubing was fitted over the female end of the connector. Three electrodes were then placed in a row and held in place while the outside wires were twisted together around the middle wire. A drop of dental cement was then used to keep the electrodes in place. Finally the wires were measured and cut so that a distance of 0.5 mm separated each wire tip from the next closest one. This resulted in an electrode assembly with a deep center electrode, intended for use as a stimulating

electrode, and two recording electrodes flanking it a different heights. The uninsulated cross-sectional area of each electrode tip was sufficient to produce a good EEG signal in all electrodes during implantation.

Apparatus

The apparatus consisted of a five foot diameter fibreglass water tank, colored white on the inside. The tank was filled daily with cold water (approx. 9 degrees Celsius) in which a mixture of skim milk powder and commercially available whey powder was dissolved, such that the water was rendered opaque. Submerged one to three cm under the surface was a plexiglass platform 12 cm square. To allow the animals to grip the platform a monofilament nylon line was randomly laced through holes drilled in the platform.

A black plastic rectangle (8 inches X 5 inches) was placed in various locations on the wall of the tank about 2 inches above the waterline. This served as a distinctive cue within the tank.

Each trial for each subject was recorded on video tape for later analysis. A video signal special effects generator was employed to superimpose an insert containing a digital clock, and identification of the trial and subject number of each trial on each video record. This allowed the latency for subjects to climb on the platform to be accurately determined during later analysis.

All electrical stimulation of the hippocampus employed a standard 120 volt Variac connected to a 1:1 stimulus isolation transformer. Two 500 MOhm resistors were placed in series with the two output leads of this transformer to produce a current of 10 microamps measured using half amplitude (peak to baseline).

All measurements of distances swum in the tank on a trial were obtained using a Apple II microcomputer graphics tablet and software developed at the University of Lethbridge, Alberta, by Sutherland and his associates.

Procedure

The procedure used during all acquisition trials was as follows. The subject was removed from its home cage which had been placed on a table close to the tank. After being held for a brief period while the experimenter turned on the video recorder the subject was carried to the entry position for that trial, and gently released, facing the tank wall. Upon completion of the trial the video recorder was turned off and the rat retrieved from the platform, and replaced in its home cage to await the next trial. During acquisition trials four or five rats were run in succession, so that each trial for each rat was separated by four or five intervening trials.

For each subject a trial lasted until the platform was reached or a maximum of 120 seconds had elapsed. During initial acquisition trials, once the platform was reached,

subjects were allowed to remain on it for 15 seconds, in order to allow a segment of the rearing behavior to be recorded for later analysis. This 15 second period was continued until the overall amount of rearing declined to near zero for all subjects. Rearing was quite distinguishable on the videotapes and was scored for the first eight trials.

Entry points for each subject on each trial (and also for the position of the cue for the MAP subjects, and the cue and platform location for the CUE subjects, after Trial 16) were quasi-randomly selected such that each subject entered from each of four potential entry points (compass N, S, E, & W), over every group of four consecutive trials. In this way it was ensured that all subjects would have equal experience with all entry points.

Although acquisition trials were conducted identically for all subjects, conditions differed slightly for each group. To begin with each group was trained on alternate days for the first 44 trials. For stimulation trials, though, all subjects were tested on the same day.

The experiment involved four groups with 10 subjects per group. These consisted of two groups, M1 and M2, which were trained to employ a map, or place, strategy, and two groups, C1 and C2, which were trained to use a guidance strategy to perform the MWT. During acquisition trials groups M1 and M2 were treated as a single group (to be

referred to collectively as the MAP group) as were groups C1 and C2 (to be referred to collectively as the CUE group). During stimulation trials, of course, one subgroup was stimulated while the remaining subgroup acted as a control group.

The major difference between the groups concerned the relationship between the platform and the black cue card over trials. For trials one to sixteen, the platform position for the MAP group was in the south-east quadrant of the pool, and the cue card was located on the south-west wall. For the CUE group both the platform and the cue card were in the south-west quadrant.

Days One and Two. All subjects were given two initial days of four trials each, since each trial tended to last close to the maximum allowed and it was felt that eight trials on these initial acquisition days was too many. Since most subjects had shown substantial acquisition of the task by the end of day two all subsequent training days consisted of eight trials per day.

During the first eight trials for each subject, three distinct behaviour patterns were recorded; rearing, scratching at the cue while swimming, and jumping off the platform once it had been reached.

Measures were obtained of the number of times a subject reared in a fifteen second period which started when a subject climbed onto the platform. A rear was

operationally defined as any movement which caused the head to rise and the body to assume a more vertical posture. Typically rears were easy to identify, consisting of overt stretching to the full height of the subject, followed by orienting movements of the head. The time spent scratching at the black cue card on the wall of the tank was recorded for each subject, as was the number of times the subject jumped back into the water from the platform. These behaviours were subsequently analysed when the video tapes were viewed.

Day Three. On Day 3 of acquisition trials all subjects were shifted to eight trials per day.

Day Five - Probe Trials. Following 16 trials under these conditions all subjects were then given a series of probe trials to ascertain the degree to which these conditions had been successful in requiring the two groups to use different strategies to perform the MWT. Probe trials for the MAP group consisted of maintaining the platform location but moving the cue card around randomly on each trial. The probe test for the CUE group involved keeping the platform and cue card together, but moving their position randomly over trials, such that the only indicator of the platform position available to these subjects was the cue card. The result of the probe trials revealed that the CUE group were relatively disrupted by these changes, indicating that some subjects were learning

to follow the cue, while others were actually learning a spatial strategy. Consequently the training procedure was changed after trial 16, so that the conditions in effect during the probe trials were maintained throughout the remaining acquisition trials.

Days Six To Eight. Each group was then given three additional acquisition days to learn the changed conditions after Trial 16.

Days Nine to Eleven - First Stimulation Days. Following acquisition the MAP and CUE groups were divided into subgroups M1 and M2 and C1 and C2 respectively. Subjects in each subgroup were matched according to their performance over acquisition training. In order to match the subjects all subjects were ranked in ascending order by the mean distance swum by each subject over the last 20 acquisition trials. In addition a similar ranking was made using the mean distance swum over the last four acquisition trials. This resulted in a record for each subject of how it had performed in general during acquisition, and specifically on the last day of acquisition. Each mean was then compared with the overall mean for that group so that each subgroup could be matched for those subjects which deviated substantially in either direction from the group means.

Stimulation trials began between the third and fourth trials on the ninth day of training (trials 47-48). After three normal trials, each subject in groups M1 and C1

received thirty seconds of stimulation (10 microamps, 60 Hz) during the latter half of the one minute interval between trials 3 and 4 (47-48). On stimulation days each subject was given eight consecutive trials, with the cue and platform positions changed as required between trials. After the single stimulation, the subject was placed in the tank for the remaining five trials for that day. In order to obtain an accurate picture of the course of recovery from stimulation, consecutive trials were run as quickly as possible. Control groups M2 and C2 were treated identically, although they, of course, received no stimulation. The above procedure was employed for a total of three stimulation days.

Days Twelve and Thirteen. Following this first part of Experiment One, all groups were given a two day rest, to allow complete recovery from any remaining effects of stimulation, prior to more stimulation trials.

Day Fourteen - Probe Trials In Tent. In order to discover whether the training conditions had been effective in producing groups which had learned to employ different strategies to perform the Morris water task, a series of four trials was given to groups M1 and C1. For these trials a cheesecloth tent was constructed, which was then placed over the tank in order to eliminate all the usual fixed distal room cues present during previous trials. The cue card was still included within the tank, since the trials

were designed to see whether the MAP group was disrupted by the loss of distal cues, and whether the CUE group performance was unaffected since the cue they had been following was still present. To ensure that all room cues were eliminated, each subject was placed under a black cloth upon removal from its cage, walked around the room, and spun around so that familiar handling cues and cues which might have been available just before entry into the pool were also eliminated. In addition the video recorder and video monitor were moved so that any auditory cues would come from a different direction for these trials. The probe results confirmed that each group had, in fact, been employing a different strategy.

Procedure - Part Two

One interesting finding which resulted from the first stimulation trials was that the stimulated C1 group subjects appeared unable to locate the platform if they swam by it on their initial approach to the card on the tank wall. The subsequent failure to find the platform suggests that the task for the CUE group contained a substantial spatial component, and that stimulation of the CUE group resulted in disruption of this component, producing large swim distances on stimulation trials. The spatial component of the cue task was due to the fact that the cue card and the platform were physically separated, since the card hung on the wall in the quadrant in which

the platform was located.

To investigate this further the spatial component of the cue task was eliminated by suspending the cue immediately over the platform, so that any subject which learned to approach the cue would automatically reach the platform when it swam under the cue. The cue employed for the remainder of Experiment One was a black cube (2.5 inches per side), suspended directly over the platform approximately three to four inches above the water. The base of the cube was hollow to allow subjects to rear up inside the cube while exploring it, thus allowing distinctive tactile cues as well as visual cues to be associated with the cube.

A secondary purpose of Part Two was to replicate the results obtained from the first three stimulation days, this time stimulating groups M2 and C2, while groups M1 and C1 acted as controls.

Days One To Four - Acquisition. Following the last trials of Part One (probe trials with tent), all subjects were given three days to rest, followed by four days of acquisition training in order to get used to the suspended cue which replaced the cue on the wall. During these days all other conditions were identical to those present immediately prior to the first stimulation days of this experiment.

Days Five and Six - M2 and C2 Stimulation. All

stimulation procedures were identical to those used previously. Stimulation was given for thirty seconds (10 microamps, 60 Herz) immediately following trial three on each day.

Days Seven & Eight - M1 and C1 Stimulation. Following the stimulation days for groups M2 and C2 two final stimulation days were held, during which only groups M1 and C1 were run. On both days each group received stimulation between trials three and four, as they had previously during the first part of Experiment One. The purpose of this additional testing under stimulation was to determine if the presence of the suspended cue would eliminate the large swim distances which appeared in the original data for the stimulated C1 group. A second purpose of this last stimulation session was to see if the M1 group again showed a stimulation-induced disruption to that of Experiment One.

Table 1 provides an overall summary of the procedures used for each group in Experiment One.

Table 1. Summary of Experiment One Procedures. Table shows chronological order of events during Experiment One for all groups.

GROUPS	1ST ACQ. DAYS	1ST STIM DAYS 1	2	3	PROBE TRIALS IN TENT	2ND STIM DAYS 1	2	3RD STIM DAYS 1	2
M1	NST	ST	ST	ST	NST	NST	NST	ST	ST
M2	NST	NST	NST	NST		ST	ST		
C1	NST	ST	ST	ST	NST	NST	NST	ST	ST
C2	NST	NST	NST	NST		ST	ST		

ST - RECEIVED STIMULATION

NST - NO STIMULATION

BLANKS - GROUP NOT RUN

Results

Electrophysiological Results

The surgical techniques employed in these experiments involved electrophysiological recording from the electrodes in each assembly as they were being lowered into the brain. According to Collier et al (1982) this makes possible highly accurate placement of electrodes into the rat dentate gyrus. When an electrode passes through the dorsal CA1 pyramidal layer the recording is characterized by a burst of high amplitude spikes. This is followed by a period of relative silence until the descending electrode reaches the molecular layer of the dentate gyrus, at which time a second burst appears which is characterized by slightly lower amplitude spikes.

All examples of physiological records are presented in Appendix One. Figure A shows a representative example of these two bursting patterns. The top trace was obtained from the electrode as it reached the CA1 pyramidal layer, while the bottom trace was obtained when the same electrode reached the molecular layer of the dentate gyrus. The figure illustrates the slightly smaller amplitude spikes which characterize the dentate gyrus records. Once the latter pattern appeared in the deepest electrode of the assembly the assembly was fixed in place.

Typically a period of relative silence followed a

burst, at the pyramidal and molecular level alike. Most likely this was due to a slight potassium block resulting from the bursting activity. When the area recovered it was common for well-defined theta to appear in the record. Figure B in Appendix One provides an example of these theta waves. The top trace shows theta waves obtained from the pyramidal layer. The bottom two traces show simultaneous traces obtained from the dentate gyrus (top) and pyramidal layer (bottom).

While the contralateral assembly was being lowered into place, stimulating current was passed into the already implanted stimulating electrode to allow an evoked potential to be recorded by the descending electrode. Figure C in Appendix One provides some sample evoked potential records obtained in this way from different subjects. It can be seen that since each record consists of multiple traces, the evoked potentials obtained were fairly consistent. Evoked potentials were obtained from the majority of subjects implanted and representative records from each electrode in the assembly are presented.

Histological Results

Following Experiment One all subjects were perfused intracardially with a saline solution followed by 10 percent buffered formalin. The brain and electrode assembly were then removed in one piece and immersed for about a week in formalin to allow sufficient time for the fixation

process. The electrode assemblies were then removed from the brains, and the brains were blocked. Standard paraffin embedding procedures were used and coronal ribbon sections 10 microns in thickness were obtained from each embedded brain. Every fifteenth section was then mounted on microscope slides and stained with cresyl violet.

The results of these procedures indicated that all placements were accurately located along the hilus of the dentate gyrus. That is, the tip of the deepest electrode (used to stimulate) was located along the hilus, while the middle and shallow electrode straddled the pyramidal layer of the dorsal hippocampus. Figure A (in Appendix Two) presents photomicrographs of some representative sections, showing clearly the electrode track ending on the hilus of the dentate gyrus. The large size of the superior portion of the electrode track is due to the fact that the three electrodes twisted together were approximately one millimetre wide. Finally some additional width was added due to the fact that the top two electrodes tended to protrude slightly from the assembly at their respective tips, as a result of being bent to produce the desired distance between the tips.

Behavioral Results

Scratching At Cue Card. For the first few acquisition trials rats from both groups would typically stop swimming

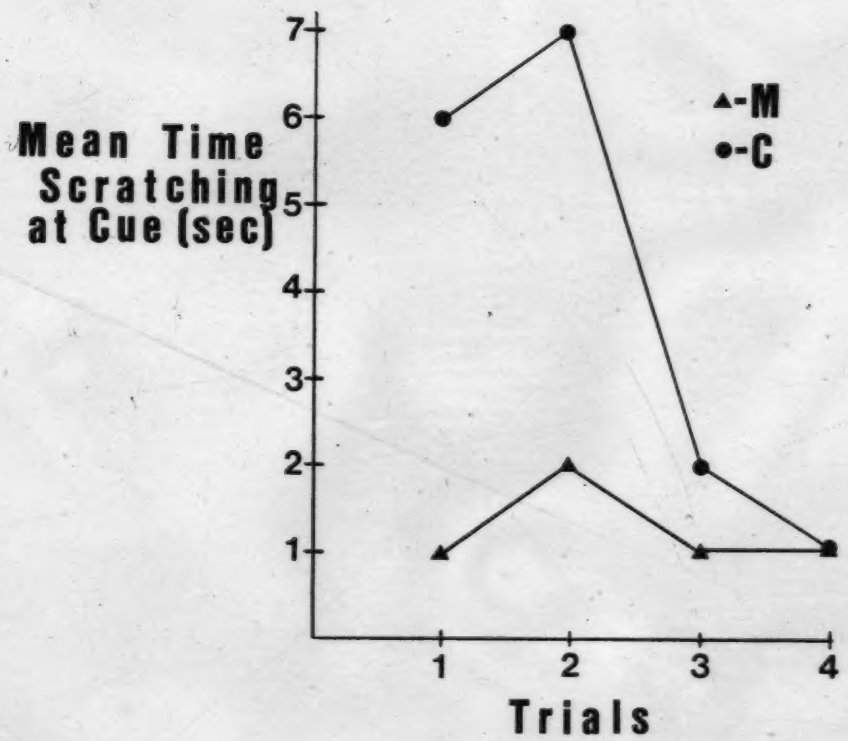
when they passed close to the black cue card, and begin to scratch at it with both forelimbs. Of interest was whether either group spent more time scratching on the average over these trials. Figure 4A presents the mean time spent scratching at the black cue card by both groups during the first four acquisition trials. The CUE group showed significantly more inclination than the MAP group to scratch at the cue over these four trials $F(1,33) = 9.84$, $p < .005$.

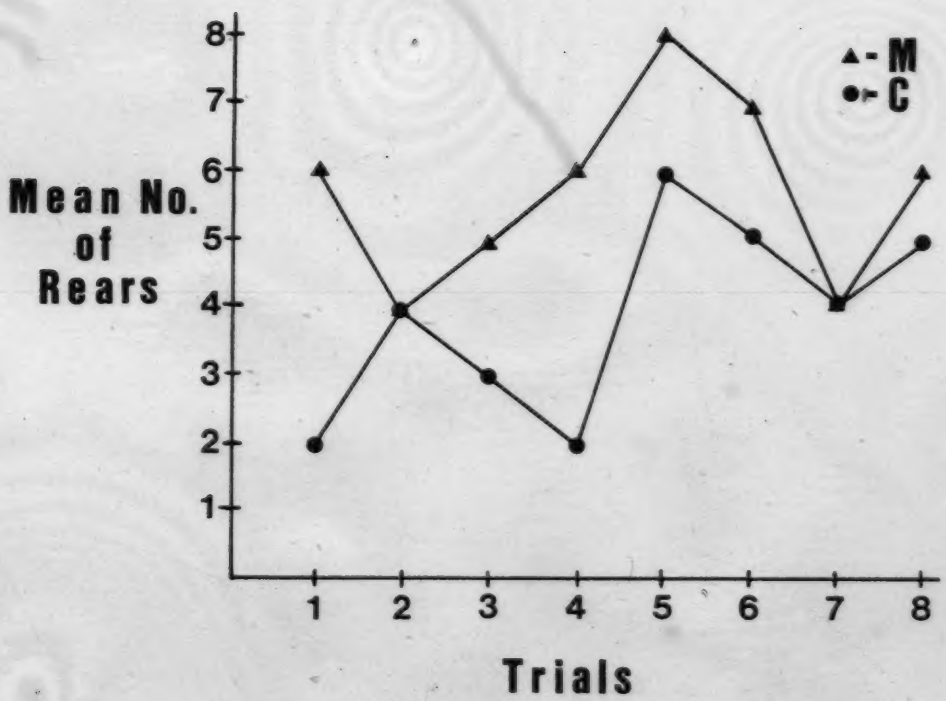
Rearing. Measures were obtained of the number of times a subject reared during a fifteen second period which started when the subject climbed onto the platform. While on the platform most subjects would stand on their hind legs, with their forelimbs held close to but out of the water. A rear was operationally defined as any movement which caused the head to rise and the body to assume a more vertical posture. Typically rears were easy to identify on the video recordings, consisting of overt stretching to the full height of the subject, followed by orienting movements of the head.

The mean number of rears per successful trial (a trial in which the rat reached the platform, climbed onto it, and remained on it for 15 seconds was considered a successful trial) is plotted for each group in Figure 4B. This figure shows that the MAP group reared significantly more than the CUE group over the first eight trials of acquisition

Figure 4. Mean Time Spent Scratching At Cue Card And Mean Number Of Rears During Acquisition.
Graph A presents the mean time spent scratching at the cue card for both groups over the first four trials. Notice that the MAP group scratches relatively little throughout the first four trials. In contrast the CUE group shows a high level of scratching initially, followed by a decline to the MAP level.

Graph B shows the mean number of rears per trial for both groups over the first eight acquisition trials. The figure shows that the MAP group reared consistently more than the CUE group throughout the first eight trials. The difference is most pronounced on the first four trials.


Mean Time Scratching at Cue (sec)
7
6
5
4
3
2
1
▲-M
●-C
1
2
3
4
Trials


Mean No. of Rears
8
7
6
5
4
3
2
1
▲- M
●- C
1
2
3
4
5
6
7
8
Trials

$F(1,13) = 19.63$, $p < .001$.

Leaving the Platform. The third behavior measured during acquisition trials was the average frequency with which each group jumped from the platform back into the water, before it spent an uninterrupted 15 seconds on the platform. It was noted that during the first few acquisition trials rats would often climb onto the platform and then jump back into the water and swim away from the platform.

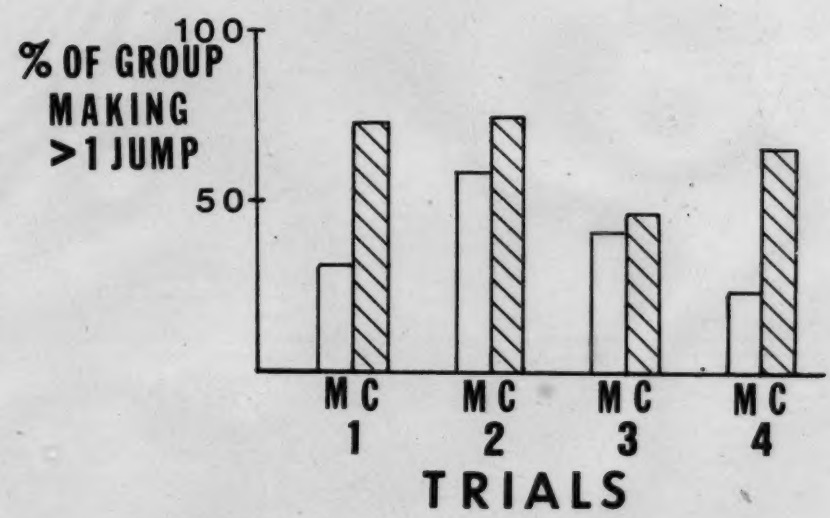
The results for this section were obtained by first determining the number of subjects in each group which successfully reached the platform on each trial (although the subject did not necessarily have to remain on the platform for the full fifteen seconds in this case). Histogram B in Figure 5 shows, for each of the first four trials, the percentage of each group that successfully reached the platform. From these results, the percentage of those (successful) subjects in each group which jumped from the platform at least once during a trial was calculated, and the results are presented in histogram A of Figure 5. This histogram clearly shows a greater incidence of leaving the platform for the CUE group over all four trials. The data represented in this figure are even more striking when considered in conjunction with histogram B. From these two histograms it can be seen that although the CUE group showed less overall success in finding the platform on the

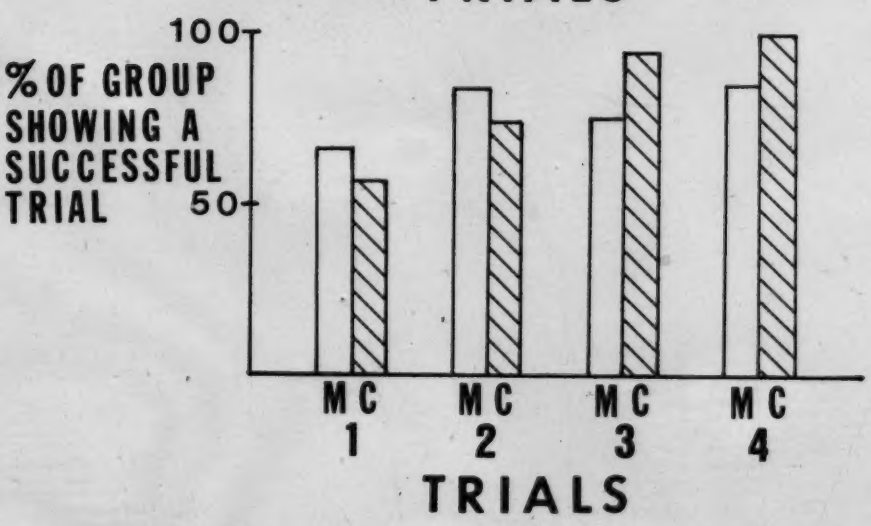
Figure 5. Figure Illustrating The Differences Between The MAP And CUE Groups In Their Tendency To Jump From The Platform.

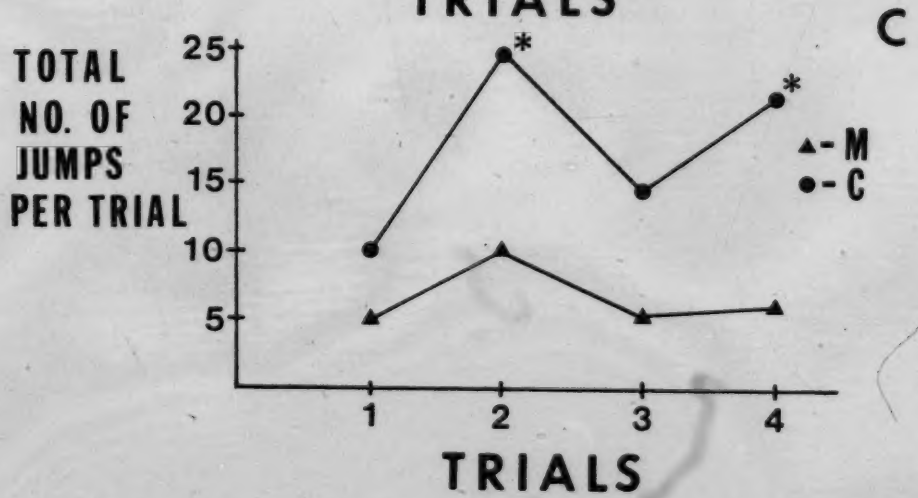
A. This histogram shows the % of subjects making at least one jump on each of the first four trials. (M = MAP; C = CUE) Note that the CUE group jumped consistently more than the MAP group.

B. This histogram shows the % of a group making a successful trial for each of the first four trials. Note that this graph shows that the MAP group started out being more successful than the CUE group, but that by trial three the CUE group was showing substantially better performance. Also note that in spite of this initial superiority the MAP group still shows fewer jumps throughout the trials.

C. This graph shows dramatically the difference in total jumps between the two groups for this time period. The CUE group consistently shows more jumps, in spite of the fact that fewer CUE subjects actually reach the platform on trials one and two.


A
100
% OF GROUP MAKING >1 JUMP
50
MC 1
MC 2
MC 3
MC 4
TRIALS


B
100
% OF GROUP SHOWING A SUCCESSFUL TRIAL
50
MC 1
MC 2
MC 3
MC 4
TRIALS


C
TOTAL NO. OF JUMPS PER TRIAL
25
20
15
10
5
*
*
▲- M
●- C
1
2
3
4
TRIALS

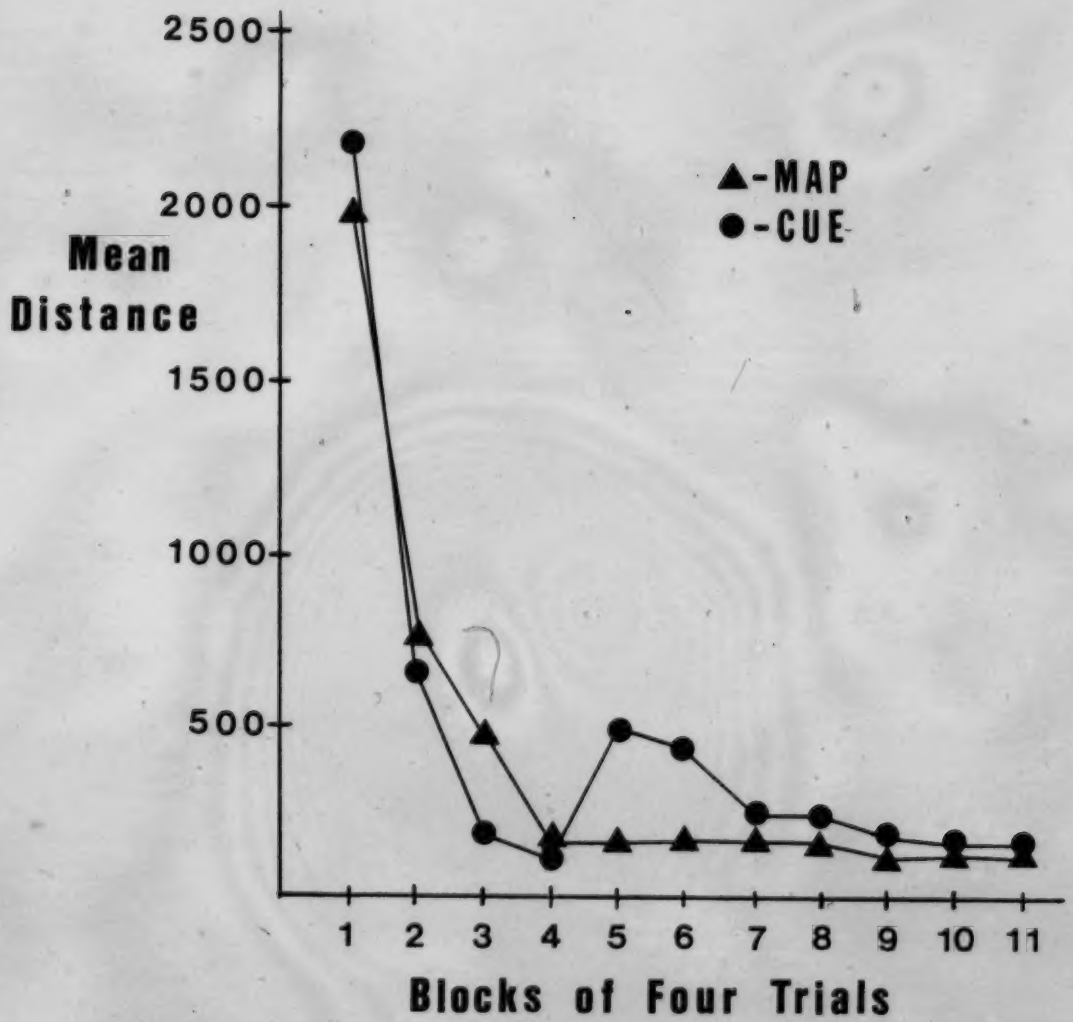
*-p < .05

first two trials, those CUE subjects which did reach the platform on any of the first four trials showed a much greater tendency to jump back into the water than corresponding MAP subjects. This point is also shown by graph C in Figure 5, which presents the total number of jumps on each trial for the MAP and CUE groups. Independent one-way analyses of variance on each trial revealed that on acquisition trials two, $F(1,29) = 5.74$, $p < .05$, and four, $F(1,38) = 6.58$, $p < .02$, the CUE group jumped from the platform significantly more than the MAP group.

Another result worth noting is that by trials three and four the CUE group showed success rates at finding the platform of ninety-five and one hundred percent respectively, in contrast with rates of seventy-five and eighty-five percent for the MAP group. Thus, although the CUE subjects left the platform more frequently, they seemed to show more rapid overall acquisition of the task of locating the platform.

Swimming Distance - Acquisition. Figure 6 presents a summary of the mean distance swum on each block of four trials for each group over all the acquisition trials. In spite of the differences in the behaviors discussed above, there was no significant main effect of acquisition strategy for distances swum by each group over the first sixteen acquisition trials, run over the first four days of training.

Figure 6. Mean Swim Distances During Acquisition.
Note that in spite of the differences in rearing, and jumping from the platform, both groups are very similar in their overall acquisition curves. It can also be seen that the CUE group was more disrupted by the introduction of the conditions for the first probe trial (Block 5) but that recovery from this was swift and complete by the end of training.

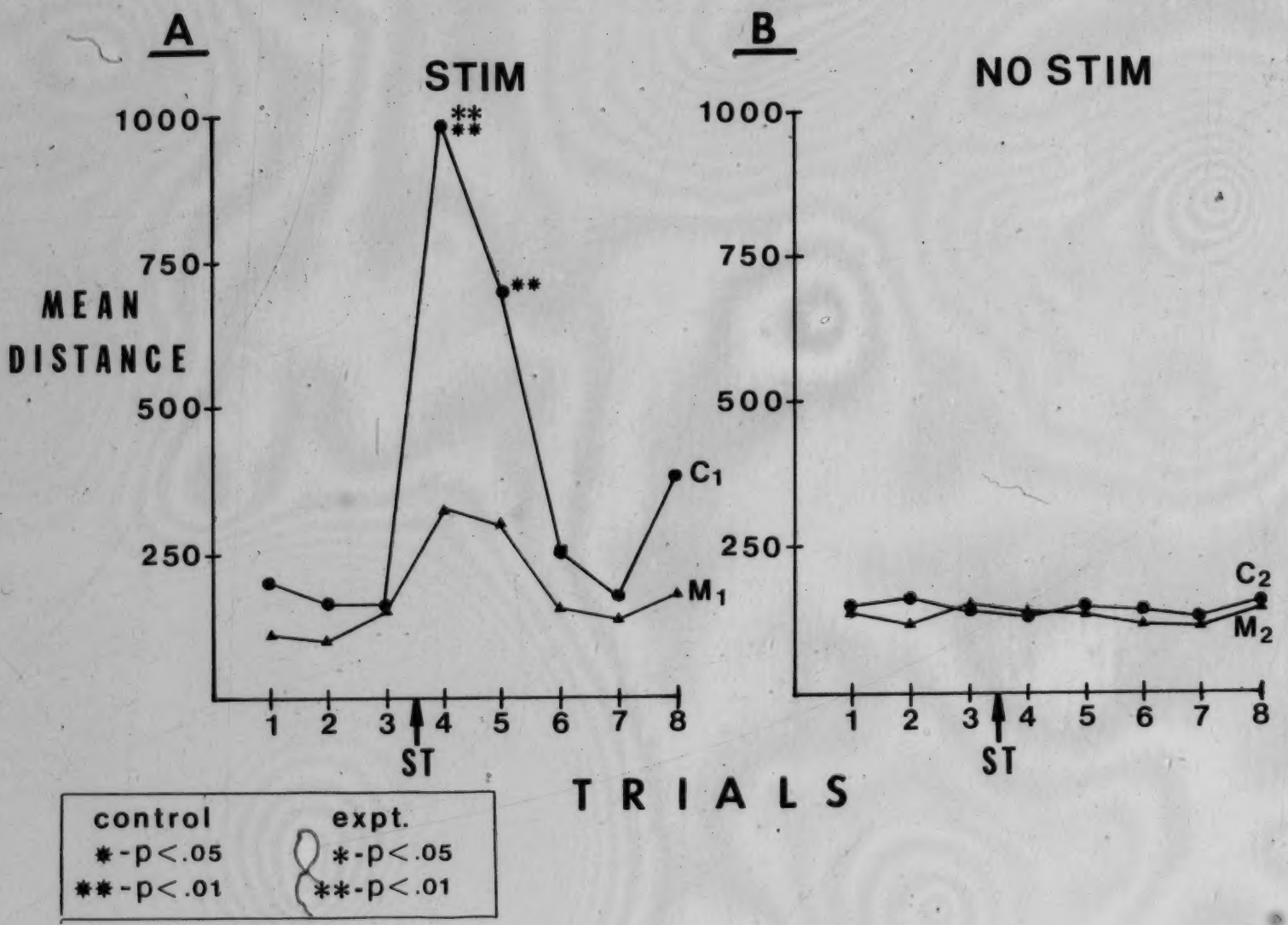

2500
2000
1500
1000
500
Mean
Distance
▲-MAP
●-CUE
1 2 3 4 5 6 7 8 9 10 11
Blocks of Four Trials

However, as Figure 6 shows, the CUE group was more disrupted than the MAP group by the change in trial conditions instituted during the initial probe period of trials 17 to 20 (Block 5). An overall analysis of variance revealed that this difference was highly significant $F(1,37) = 14.61$, $p < .0005$.

It will be remembered that the change at this point involved moving the platform location for the CUE group, but not the MAP group. As a result of this finding, the probe trial conditions were maintained for the remaining acquisition trials, until a stable baseline was obtained for both groups.

Stimulation Days One To Three. The effect of stimulation on groups M1 and C1 is presented in Figure 7. This figure shows a dramatic, but transient, effect of stimulation which is strongest for both groups on trial four. Figure 7 presents a graph of the significant three way stimulation by strategy by trials interaction $F(7,245) = 2.45$, $p < .02$ obtained from the overall analysis of variance of the first three stimulation days. This figure shows the mean swim distance for each trial for stimulated groups M1 and C1 (graph A) and their corresponding non-stimulated controls M2 and C2 (graph B), collapsed over all days. The reader is reminded that stimulation occurred only once on each day, between trials three and four. As the figure clearly shows both stimulated groups were disrupted,

Figure 7. Graph Of The Significant Three-Way Interaction Obtained For The First Three Days Of Stimulation.
The graphs show the interaction means for each group over eight daily trials for stimulated and non-stimulated subjects. Each graph represents the data for three days combined. It can be seen that stimulation (arrow following trial 3) had a dramatic, but transient, effect on groups C1 and M1, while the corresponding control groups C2 and M2 did not differ.


A
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B
NO STIM
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750
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MEAN DISTANCE
**
**
**
C1
M1
C2
M2
1 2 3 4 5 6 7 8
ST
TRIALS
control
expt.
*-p<.05
**-p<.01
*-p<.05
**-p<.01

when compared to their non-stimulated control groups in graph B. It is also clear from graph A that the effect of stimulation seems to have been much greater on the CUE group than the MAP group, a somewhat unexpected finding. Independent t-tests revealed that on trial four group C1, $t(490) = 5.41$, $p < .001$, was significantly impaired with respect to its appropriate control. In addition group C1 was also highly significantly different from group M1, $t(490) = 4.26$, $p < .001$. (The symbols indicating the level of significance for individual points in Figure 7 require some explanation. Since it is of interest to compare each experimental group with its own control group, and also each experimental with the other experimental group, two different symbols are required. The eight-pointed star indicates that the group is different from its control at the $p < .05$ level. The asterisk indicates that the experimental groups differ at the $p < .05$ level. If two identical symbols are present the difference is significant at the $p < .01$ level. This scheme is employed throughout the remaining relevant figures in Experiments One and Two.) Similar results were obtained for t-tests on trial five. Group C1 was still significantly different from group C2, $t(490) = 3.50$ $p < .001$, but no longer different from group M1.

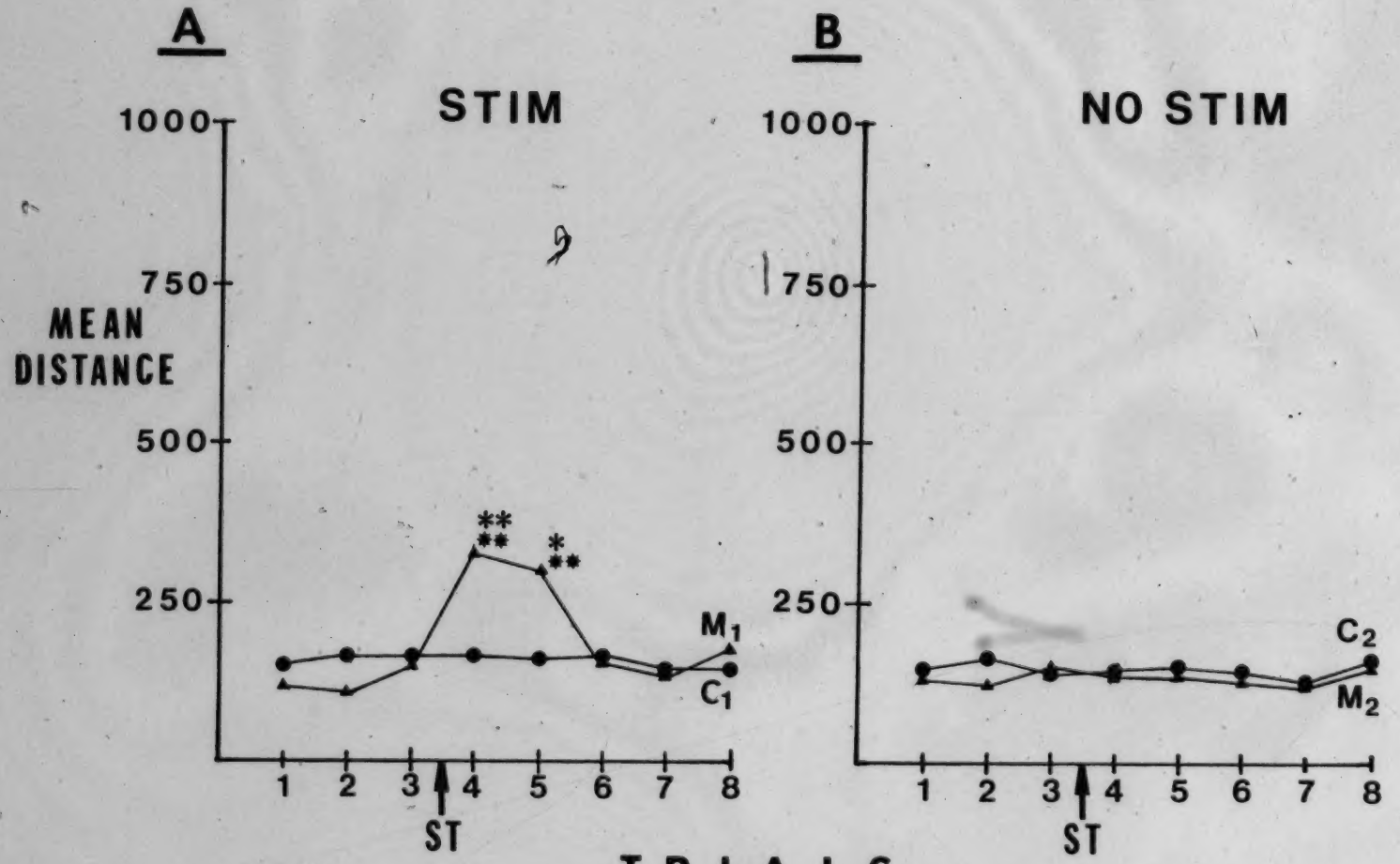
Finally one result worth emphasizing is that the stimulation effect is quite transient, having declined

substantially by trials six or seven.

Analysis of Rescored Data. Close re-examination of the video taped stimulation trials revealed an interesting and important finding. On every post-stimulation trial in which a CUE subject was disrupted, the animal would swim directly toward the cue card when introduced into the tank. However, since the platform was physically separated from the cue card hanging on the wall, if the subject swam past the platform on its way to the cue card, it would then begin to swim around the circumference of the tank, thus consistently missing the platform and producing large swim distances on these trials. This behavior suggested that perhaps an experimental artifact, namely a spatial component involved in locating the platform with respect to the cue card, was responsible for the elevated scores of the CUE subjects. Since these subjects were trained to approach the cue card, it appeared important to assess whether they continued to approach the cue with no hesitation after stimulation. Thus the data were rescored such that the distance covered before the cue card was reached, rather than the platform itself, was recorded, and subsequently analysed.

The results of this rescoring procedure are presented in Figure 8, which shows the same three-way interaction as Figure 7 for the rescored data. (To begin with it is important to point out that the taped trials of all the

Figure 8. Graph Of Significant Three-Way Interaction Obtained Using Rescored Data For The First Three Days Of Stimulation.
These graphs are similar to Figure 7 except that in this case rescored data was employed in the overall analysis. Note that the effect of stimulation on group C1 group has completely disappeared while the effect for group M1 is unchanged. Also note that now group M1 is significantly impaired with respect to group C1 on trial four. Finally the control subgroup results are unchanged.


A
STIM
B
NO STIM
1000
750
500
250
MEAN
DISTANCE
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**
**
**
M1
C1
C2
M2
1 2 3 4 5 6 7 8
ST
TRIALS

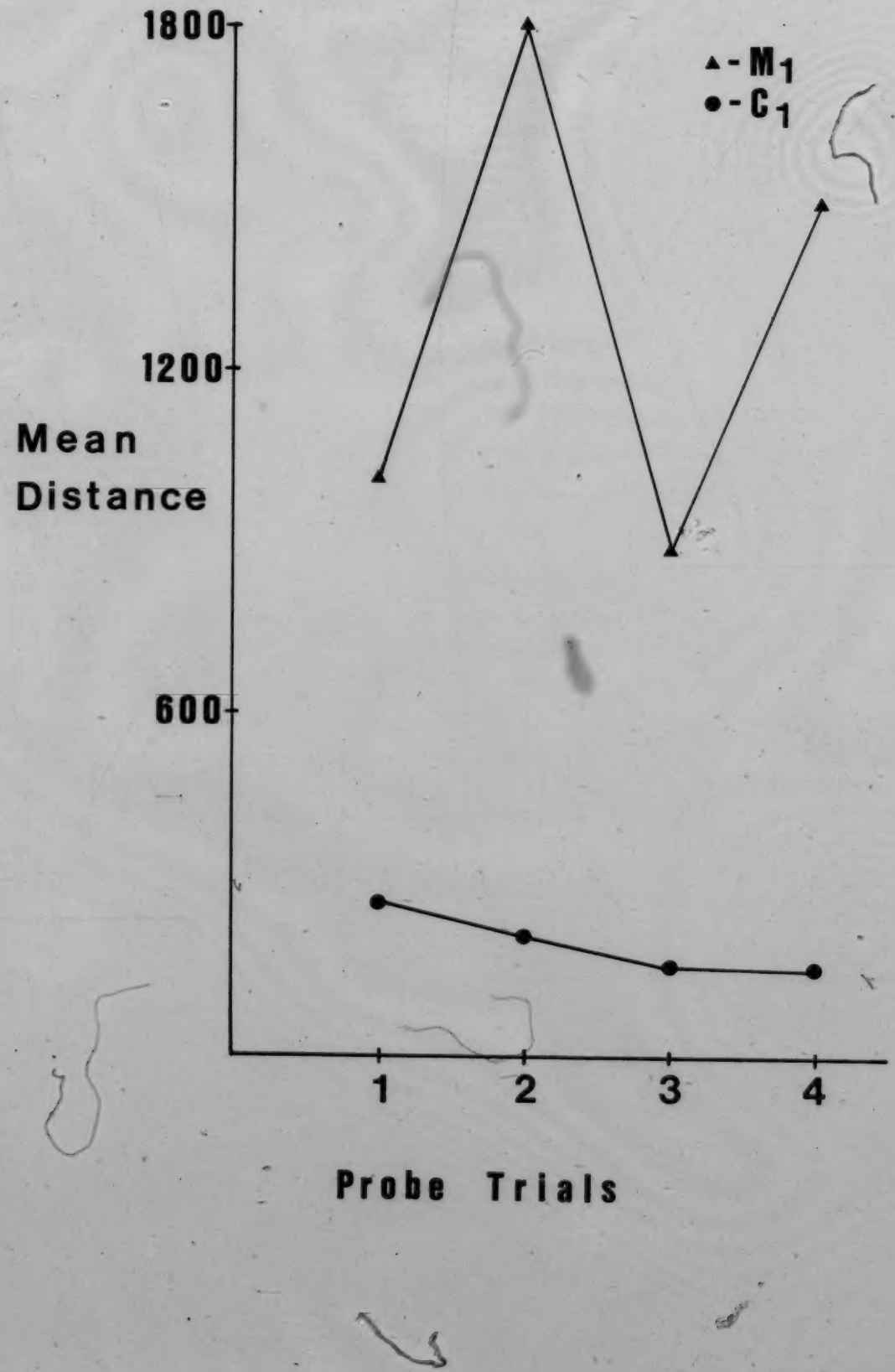
subjects in all groups were rescored in the way described above, yet the only group in which any scores were changed was the CUE group.) As Figure 8 shows rescoring the data completely eliminated the stimulation effect for group C1. Rescoring the data also maintained the overall level of significance for this three-way interaction $F(7,45) = 2.53$, $p < .02$.

Independent t-tests for trial four using the rescored data revealed that group C1 was no longer significantly different from group C2. However, eliminating the large variance introduced by the elevated scores for group C1 revealed a significant difference between groups M1 and M2, $t(490) = 3.22$, $p < .01$, which had previously been masked. Also groups M1 and C1 were significantly different on trial four, $t(490) = 2.85$ $p < .01$. On trial five similar results were found. Groups M1 and M2 remained significantly different, $t(490) = 2.89$, $p < .01$, and groups M1 and C1 were also significantly, although slightly less, different, $t(490) = 2.24$, $p < .05$.

Thus the rescored data still shows a strong transient effect of stimulation for the MAP group, but the CUE group effect has now disappeared.

One final point worth making regarding the first three days of stimulation is that there was no main effect of days, nor were there any significant interactions involving the days factor.

Figure 9. Mean Distance Swum On Each Probe Trial Conducted In The Cheesecloth Tent.
The graph clearly shows the dramatic disruption produced in group M1 by removing the fixed room cues during these trials. Although quite variable, the deficit does not appear to decrease substantially. In contrast group C1 was relatively unaffected by these measures and continued to approach the cue card when placed into the water.

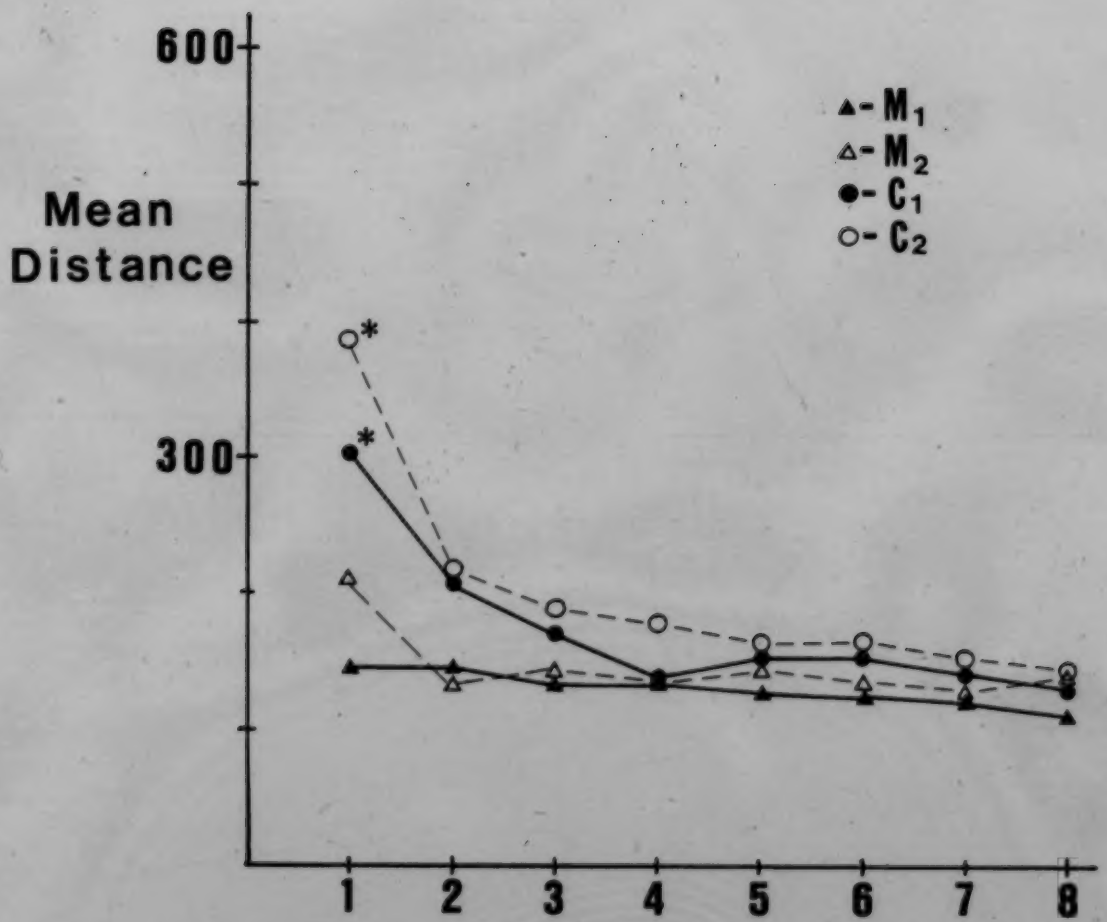

1800
1200
600
Mean
Distance
▲- M1
●- C1
1
2
3
4
Probe Trials

Probe Trials With Cheesecloth Tent. Figure 9 presents the results of the tent probe trials, which were designed to determine whether group M1 required familiar fixed room cues to locate the platform while group C1 needed only to approach the cue card. It can be seen that with no cues available to them, MAP subjects were highly disrupted in locating the platform, while the CUE subjects were relatively unimpaired, thus confirming that the groups had learned different strategies to locate the platform. The overall analysis of variance on these probe trials revealed a highly significant main effect of strategy employed to reach the platform, $F(1,18) = 68.88$ $p < .0001$.

Acquisition Trials With Suspended Cue. Figure 10 presents the mean distances swum by all groups on each block of four additional acquisition trials after the hanging cue had been introduced. The reader is reminded that for these trials the cue signalling the platform location for groups C1 and C2 was a black cube suspended over the platform. Figure 10 shows that the CUE subjects were initially disrupted by this change, while the MAP subjects were relatively unaffected. Also it can be seen that by the end of the first day of training a substantial amount of recovery had occurred.

Analysis of the first eight trials of Experiment Two revealed that the CUE subjects swam significantly longer distances ($F(3,34) = 11.91$, $p < .0001$) than the MAP

Figure 10. Mean Distance Swum By All Groups Over All Acquisition Trials With Suspended Cue.
The increase distance seen in the early trial blocks is due to the introduction of suspended cues for this experiment. It can be seen that the greatest effect was on group C2. However by the end of these acquisition trials all the groups were showing a stable baseline.


600
300
Mean
Distance
▲- M1
△- M2
●- C1
○- C2
*
*
1
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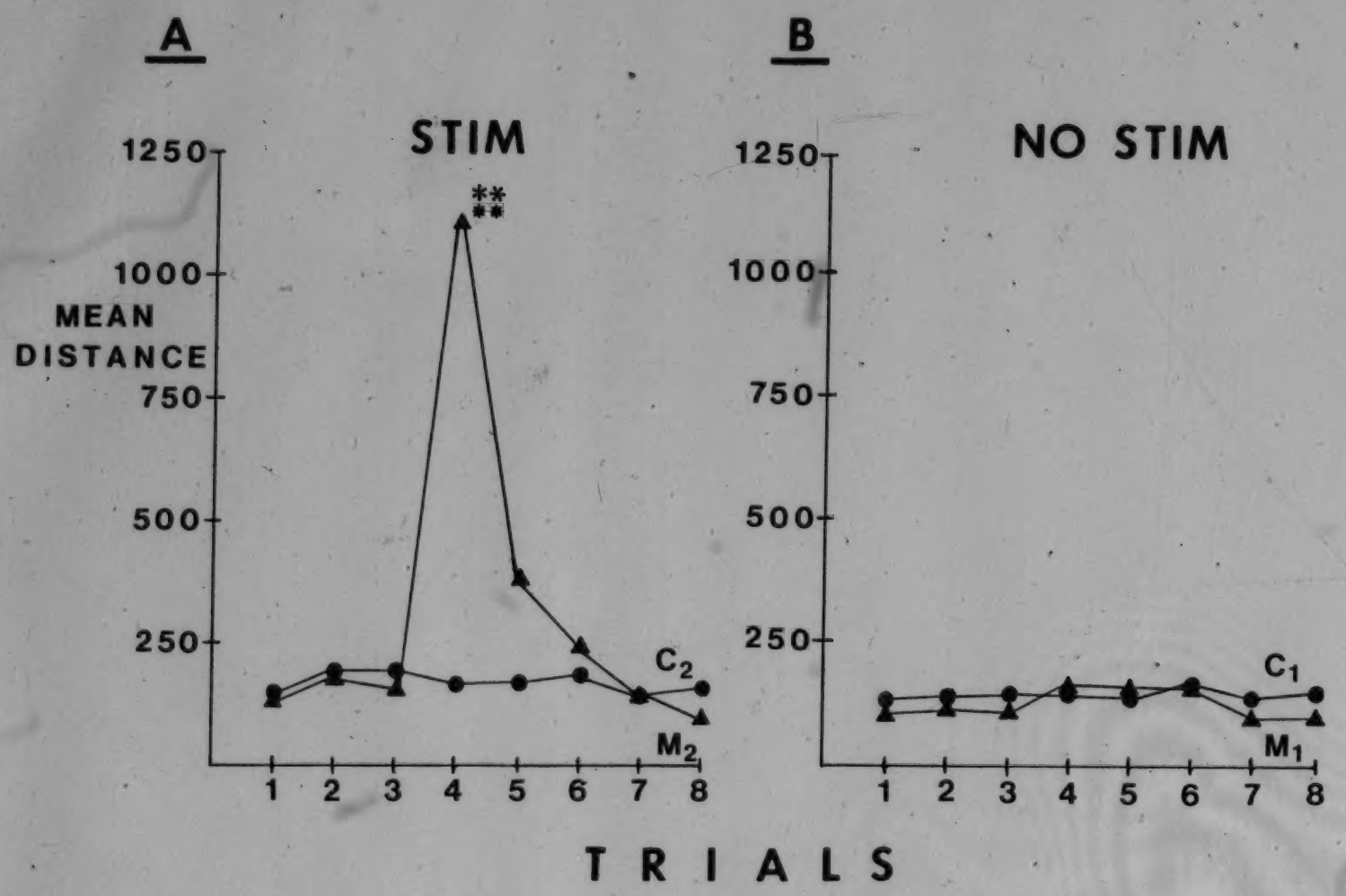
7
8
Blocks of Four Trials

subjects. Independent t-tests on the one-way analysis of variance for the first block of four trials, revealed that group C2 showed a significantly longer average swim distance than group M2, $t(238) = 2.50$, $p < .02$, and that group C1 showed significantly longer average distances than group M1 $t(238) = 2.19$, $p < .05$. There were no differences within the groups for each strategy (i.e., between groups C1 and C2, and M1 and M2). A similar analysis of the last eight training trials shows no significant difference among any of the groups, indicating that all had reached a stable baseline prior to stimulation trials.

Stimulation Trials for Groups M2 and C2. Figure 11 is similar to Figures 7 and 8 in that it presents a graph of the highly significant three way stimulation by strategy by trials interaction $F(7,245) = 5.66$, $p < .0001$ obtained from the overall analysis of the stimulation trials data. This figure clearly shows that the stimulation had a dramatic, but once again transient, effect on group M2 only, while groups C1 and C2 did not differ. As was the case for the previous results of the stimulation trials for groups M1 and C1, the data represented in this figure is collapsed over days.

Independent t-test for trial four revealed that group M2 was highly significantly different from group M1, $t(245) = 8.35$, $p < .001$, and from group C2, which was also

Figure 11. Graph Of The Significant Three-Way Interaction Obtained From Stimulating Groups M2 and C2.
Once again the graphs represent the data collapsed over days. It can be seen that the results for these stimulation days are similar to those in Figure 8. Group C2 did not differ from its non-stimulated control C1, while group M2 was highly disrupted on trial four by the stimulation. The transient nature of the effect is again evident in this graph.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C2
M2
C1
M1
1 2 3 4 5 6 7 8
TRIALS

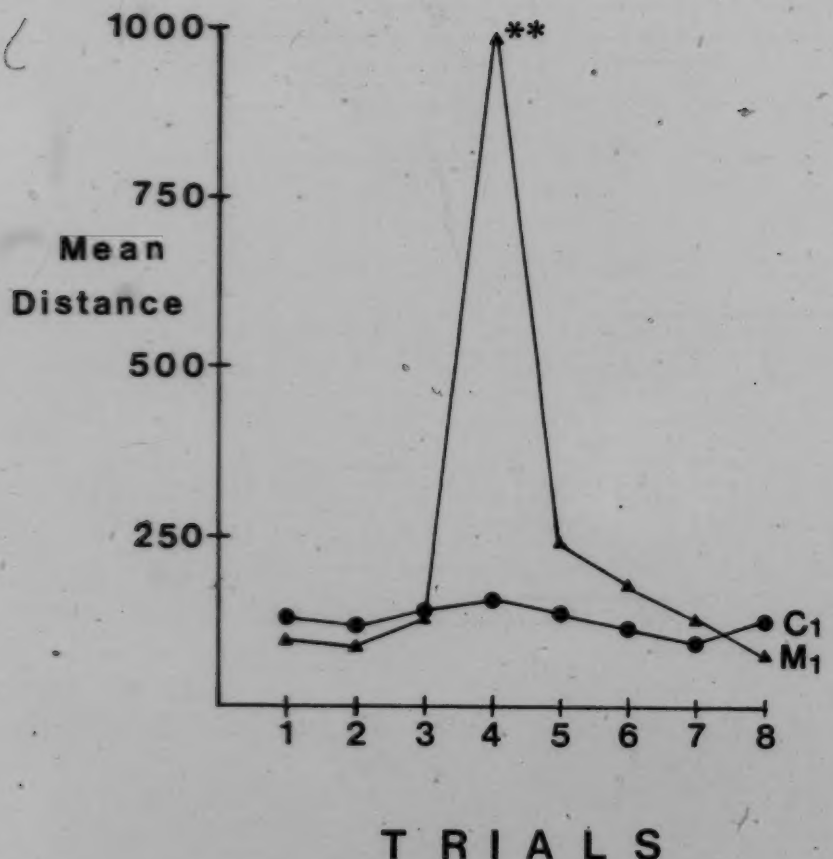
stimulated, $t(245) = 8.40$, $p < .001$. The results for trial five revealed no significant differences among the groups indicating that the effect of stimulation was only temporary. Once again no main effect for days was obtained, nor were there any significant interactions, in which days was a factor.

Second Stimulation Trials For Group M1 and C1. Figure 12 is a graph of the significant trials by strategy interaction $F(7,126) = 4.33$, $p < .0001$ which resulted from the overall analysis of the last part of Experiment One, in which only the MAP and CUE groups were run. The figure shows a strong, transient effect of stimulation for group M1, and no effect for group C1. Independent t-tests showed that group M1 swam significantly longer distances than group C1 did on trial four $t(126) = 3.51$, $p < .001$, but not on any subsequent trial. Once again, no days factor was significant, indicating consistent results over both days.

Finally it is important to compare the results shown in Figure 12 and Figure 7 for groups M1 and C1. It can be seen that the introduction of a cue hanging directly over the platform for groups C1 and C2 eliminated the disruptive effect of stimulation for these groups, while the effect on groups M1 and M2 was unaltered.

Consistency of Stimulation Effect. It has been shown in the histology section that the overall accuracy of the electrode placements was quite good. At this point it is of

Figure 12. Graph Of The Significant Two-Way Interaction Obtained From The Second Stimulation Of Groups M1 and C1.
The graph shows the effect of stimulation on groups M1 and C1 collapsed over both stimulation days. The disruptive effect on group M1 is evident in this graph. Also it is important to compare the results in this figure with those in Figure 7 for groups M1 and C1. With the suspended cues present, the increased distances seen in Figure 7 for group C1 are no longer present, while the disruption effect for group M1 is more dramatic.


1000
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Mean
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C1
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TRIALS

interest to examine the consistency of the stimulation effect, within and across subjects, to provide some further support for the conclusion that the placements were accurate. Since only the MAP subjects showed any effect of stimulation, this section is primarily devoted to them.

In Experiment One each subject in group M1 received stimulation a total of five times, while the M2 subjects received it only twice. Table 2 provides a summary of the proportion of these times in which a subject was impaired by the stimulation. To determine whether the subject was impaired on a given stimulation day, its distance swum on each trial was examined and if it was more than 150 over the baseline (ie. about 300 or over) that trial was scored as showing impairment. It can be seen from the table that every M1 and M2 subject showed impairment at least once during the course of stimulation. Also the overall average percentage of impaired trials was quite high for both groups. Lastly no subject in the M1 group showed less than two separate days of impairment, and only one of these failed to show any impairment during the first three stimulation days.

Table 2. Summary Of The Proportion Of Stimulation Trials In Which M1 And M2 Subjects Showed Impairment.

		STIMULATION DAY					
GROUP	SUBJECT	1	2	3	4	5	TOTAL
M1	1	NO	YES	NO	NO	YES	2
	2	NO	NO	NO	YES	YES	2
	3	YES	YES	YES	YES	YES	5
	4	YES	YES	YES	NO	YES	4
	5	YES	YES	YES	YES	YES	5
	6	YES	YES	YES	NO	YES	4
	7	YES	YES	NO	NO	YES	3
	8	YES	NO	YES	NO	YES	3
	9	YES	NO	YES	YES	YES	4
	10	YES	YES	YES	YES	YES	5
						TOTAL....37	
					PERCENT AVERAGE....74 %		
M2							
	1	YES	YES				
	2	YES	YES				
	3	YES	YES				
	4	YES	NO				
	5	YES	YES				
	7	YES	YES				
	8	YES	YES				
	9	YES	NO				
						TOTAL....14	
					PERCENT AVERAGE....88 %		

Discussion

The acquisition results reported indicate that although the overall rate of acquisition of the two tasks did not differ for the two groups, nevertheless there were some subtle differences apparent in the way each group acquired the task. For example, the decreased amount of rearing shown by the CUE group suggests that these subjects did not find it as necessary to familiarize themselves with the arrangement of fixed objects outside the tank. This implies that the presence of the single fixed cue within the tank was sufficient for this group to locate the platform. In contrast the cue card could simply have been another distant fixed cue for the MAP group, since it was located across the pool from their platform location. In fact there were several outside objects which were equally close to the MAP group platform location, which could serve equally well as landmarks for this group.

The suggestion that the cue card was the most important cue available for the CUE group is further supported by the finding that this group had a much greater tendency to scratch at the cue card on the tank wall. This indicates that the card had become much more important for the CUE group, perhaps because they had quickly learned to find the platform once they had reached the cue. In contrast, it would have been detrimental for the MAP subjects to have spent time scratching at the cue since

their platform location was quite distant from it.

Finally the greater tendency of the CUE group to leave the platform is also interesting. Typically CUE subjects would reach the platform and then quickly jump off it and swim off in some other direction. Then they would return and climb back onto the platform. One of the striking aspects of this behavior is that the subjects appeared to have no difficulty locating the platform after the first one or two trials. This ease of locating the platform is reflected in the finding that the success rate for reaching the platform on trials three and four was 95% and 100% respectively for the CUE group and only 75% and 85% for the MAP group.

It is suggested that it may be possible to regard these differences as different manifestations of the same underlying phenomenon. Perhaps the CUE group learned quite quickly that the cue card was all they required to reach the platform, whereas the MAP group needed time to become familiar with the whole constellation of available cues and the relationships among these cues. The disparity in time spent scratching at the cue card provides additional support for this interpretation. If this was the case, then the CUE subjects might be expected to explore the tank more fully, since they could quickly reach the platform without difficulty by simply swimming toward the cue card. This would then account for the increase in jumping from the

platform seen in this group. It also suggests a reason for the MAP group showing substantially less jumping, since once the platform was reached subjects tended to remain on it and rear frequently, and in different directions, presumably to learn to recognize the various landmarks which could guide them to the platform in future. Thus this interpretation would predict that the MAP subjects would show more rearing, less jumping from the platform, and less scratching at the cue card.

The difference in swim distance following the condition changes on probe trials 18 to 20 indicate that the fixed nature of the cue card and platform for the CUE group allowed a substantial spatial component to enter into the task for this group. Suddenly moving the platform and cue card combination had a disruptive effect on the CUE group, which can be seen in Figure 6. Thus at this point in the acquisition trials it was found that some CUE subjects were employing a spatial strategy in addition to a guidance strategy. For this reason the probe trial conditions were maintained until the end of this and subsequent experiments. The remaining trials in Figure 6 show that the CUE group soon learned to solve the task with these new conditions, so that when stimulation trials were given, there was a fairly high degree of confidence that the two groups were using the desired different strategies. The probe trials with the cheesecloth tent (discussed more

fully below) also supported this conclusion.

The results of all the stimulation trials in Experiment One strongly support the spatial map hypothesis advanced by O'Keefe and Nadel (1978). Electrically induced disruption of ongoing hippocampal neural activity produced a consistent severe impairment for subjects which were trained to use a mapping strategy to reach the platform. In contrast, subjects using a cue strategy to reach the platform were unaffected by the stimulation. As described above, the spatial map hypothesis predicts that the subjects in the MAP group, which rely on spatial cues to navigate, will be impaired by the stimulation, while the CUE group subjects, which use a simple guidance strategy, will not be impaired by the stimulation.

Although group C1 did show long swim distances following stimulation (see Figure 7), it was found that these subjects were unimpaired in their ability to swim toward and reach the cue card immediately upon being placed into the water. The increased scores for this group were apparently due to the presence of a spatial component in the cue task. Consequently rescoring the data in the manner described showed that the ability to approach the cue card had been preserved in stimulated subjects, thus the stimulation did not appear to affect the guidance strategy capacity of the CUE subjects.

In contrast there was a severe impairment of the MAP subjects following stimulation. This result remained following rescoring of all stimulated trials, and was also present on each stimulation day, indicating that the effect was fairly consistent.

The first question to ask concerning these results is whether, in fact, the experimental manipulations were successful in producing groups which were forced to adopt a map or cue strategy to locate the platform on each trial. The results of the probe trials conducted in the cheesecloth tent (see Figure 9) speak rather eloquently to this point. These trials clearly show that when all fixed distant room cues were eliminated, or relocated, and the black cue card remained present, the MAP group was totally unable to find the platform quickly and efficiently. In most cases subjects in this group reverted to swimming randomly around the tank, staying six to ten inches from its perimeter. In contrast the CUE subjects had no difficulty in quickly finding the platform under these conditions. Note that in Figure 9 the mean distances swum on each trial for the CUE group are in the same low range as those obtained for stimulation trials.

One anecdotal discovery is of interest at this point. The complexity of the constellation of cues available to the subjects under normal conditions is clearly demonstrated by the elaborate procedures eventually

required to eliminate these cues. It was quickly found that it was not sufficient to simply eliminate all room cues visible from the water by covering the tank with the cheesecloth tent. It was also necessary to relocate the video recording equipment in another room in order to cause the noise it made to come from a different direction. In addition it became necessary to eliminate any cues that the subjects may have habitually obtained from the handling procedures used. This was discovered when the first two MAP subjects were given the first probe trial with the tent. These two subjects were simply removed from the home cage and placed into the tank through an opening in the tent wall. Both subjects then quickly found the platform with little difficulty, indicating that it was not sufficient to remove only those cues available from inside the tank. This discovery made it necessary to devise the rather elaborate handling technique described in the Procedures section, including covering the subject in a black shroud and spinning it randomly around the room prior to introducing it into the tank. (The importance of these handling cues to the MAP subjects will be discussed further below.) Thus there was a high probability that during the probe trials all relevant cues had either been removed or drastically altered, except for those present inside the tank. The crucial finding, of course, is that these elaborate precautions affected only the MAP group, which

invites the conclusion that the two groups did, in fact, use different strategies to locate the platform, and that these strategies may be safely described as map and cue strategies. Further it strongly supports the conclusion that the hippocampal stimulation impaired the ability to use a map strategy, but not a cue strategy.

Having reached this conclusion, it becomes of interest to speculate briefly on the nature of the MAP group impairment. While the data obtained from these two experiments do not specifically address this issue, it may be possible to approach the problem obliquely by examining in detail the differences in the task requirements for both groups. These differences may be summed up as follows:

1. The platform position was fixed for the MAP group and randomly placed in one of four locations for the CUE group.
2. The black card (or cube) only was neither necessary nor sufficient to allow the MAP group to locate the platform. In contrast, it was essential for the CUE group to perform efficiently.

Aside from these differences, all remaining procedural steps were identical for both groups.

Since the platform location varied randomly over trials for the CUE subjects, the only way for them to find the platform immediately on entry to the water would be to

approach the suspended cue. Under these conditions, the presence or absence of other more distant fixed cues would be largely irrelevant. In fact it is difficult to conceive of any way in which the CUE subjects might profitably use these fixed cues. Thus it can be safely concluded that the task facing the CUE subjects was most easily solved by following a single salient cue on each trial. In order to conclude that this is the crucial element responsible for the different effect of stimulation on both groups, it remains to be shown that the MAP subjects were also not following a single cue, but that they required two or more cues to navigate to the platform.

To begin with it is certainly possible that MAP subjects could reach the platform simply by swimming from each entry point toward a single landmark which was directly in line with the platform. Thus it could be said that in some sense the MAP subjects were doing the same thing as the CUE subjects, ie. following a salient (to them) cue. However closer examination of this suggestion reveals that it is highly oversimplified. Upon entry to the tank, the MAP subjects, if they habitually swim toward a distant fixed landmark, must first decide which landmark is appropriate. Put another way they must be able to recognize where they are, since the choice of which landmark is appropriate depends entirely on the starting point for that trial. Thus in order to choose the correct landmark to

follow the MAP subjects must be able to recognize the landmarks which distinguish the different entry sites. The probe trials with the cheesecloth tent provide ample evidence that perception of the entry point landmarks are essential for the MAP subjects, while they are not required at all by the CUE subjects. It is suggested that this may be where the crucial difference lies between the two strategies used in the task. The CUE subjects do not require any landmarks present, either before entry or during swimming, except the single suspended cue. This cue is easily visible from all points in the pool, and, more importantly, it is possible to reach the platform from any point in the pool by simply approaching the cue. In contrast, the MAP subjects, even if they use only a single distant landmark for each entry point, must be able to identify at which entry point they are located. Thus MAP subjects do require the presence of identifying landmarks not only during swimming, but also prior to entry. Thus in order to become familiar with the tank and its surrounding environment some neural memory system, into which this spatial information may be entered, is required by the MAP subjects but not by the CUE subjects. It is suggested that this is where the spatial map requirement of the task is to be found. As the O'Keefe and Nadel (1978) hypothesis suggests, interference with this map will produce impaired capacities to navigate. The experiments described have

shown that disruption from two sources can result in impaired navigational ability. First electrical stimulation of the hippocampus has been shown to consistently disrupt spatial abilities, and second, the tent probe trials have disrupted spatial ability by not allowing the subject to perceive any landmarks to compare with the stored map. Neither the stimulation nor the tent probe trials produced disruption of the CUE subjects since they required no map at any time.

The results of Experiment One have been shown to strongly support the spatial map hypothesis of O'Keefe and Nadel (1978). Not only were stimulated subjects which required familair landmarks impaired, but stimulated subjects which simply followed a single salient stimulus were unaffected, results which are equally consistent with the spatial map predictions.

Having reached this conclusion, it becomes of interest to see how the spatial map hypothesis fares when directly compared with a competing hypothesis of hippocampal function, the working memory hypothesis advanced by Olton and his associates. Experiment Two was designed to use identical arrangements of physical stimuli in order to directly investigate the opposing predictions made by these two major hypotheses.

EXPERIMENT TWO

In contrast to the spatial map hypothesis of O'Keefe and Nadel (1978) Olton and his associates have proposed a second hypothesis suggesting a pure memory function of the hippocampus (Olton & Papas, 1979; Olton, Becker, & Handelmann, 1979). They suggest that tasks may be analysed into working memory and reference memory components. In their lengthy review of a number of earlier experiments carried out in their lab (Olton et al, 1979) Olton and his colleagues describe these two terms, drawing heavily on Honig's earlier definition (Honig, 1978).

Working memory procedures refer to aspects of a task in which stimulus information is useful for some portion of the experimental period, usually a single trial. Reference memory procedures, on the other hand, are those in which information required for a single trial, is required for all trials. Perhaps the easiest way to clarify this distinction is to study a brief example of a task regarded as having working and reference memory components alike.

The radial maze has been extensively used as a task involving reference and working memory procedures. In some of the studies described by Olton et al (1979) an eight arm radial maze was used. Typically each arm of the maze would be baited and the subject would be allowed enter any of the arms to obtain the food reward it contained. The optimum strategy for a subject to use in this task is to enter each

arm only once, thus obtaining all the rewards with a minimum of effort. This strategy is often referred to as a 'win-shift' strategy. In order to achieve this, the subject must be able to recall which arms it has entered during a trial, so that it does not waste time and energy by reentering one of the arms. Since all the arms are baited at the start of each trial, the information about which arms were entered, and the order in which they were entered, on the previous trial is irrelevant on subsequent trials. Thus information gained on a single trial is useful for that trial only, and this component of the task is referred to as a working memory procedure. From this description it can be seen that it must be hypothesized that working memory processes involve the capacity to catalogue events temporally. In this respect the concept is similar to that of episodic memory proposed by Tulving (1972). The reference memory procedures in the same task include the knowledge that all arms are baited on each trial, and that the subject is only allowed a certain number of entries into arms (usually eight) on each trial. Thus it can be seen that any stimulus information which remains constant over trials may be regarded as a reference memory component, while stimulus information which changes over trials may be regarded as a working memory component.

At this point a short digression concerning the terminology employed in the literature is of some value.

The term working memory has come to have different meanings according to the discipline of psychology which is using it. For example the term "working memory" is taken by most cognitive psychologists to be almost synonymous with "consciousness", that is, information which may be kept immediately available in a working memory store by rehearsal. In essence the concept is roughly equivalent to the Waugh and Norman (1965) concept of "primary memory". The important property of this memory store is that its current contents are lost when the subject is sufficiently distracted. In this sense "working memory" is characterized as highly labile and is usually of short duration. In contrast the term "working memory" when used by Olton et al (1979) or Honig (1978) differs in a few important ways. First the contents of working memory are not necessarily lost due to distracting events. Since subjects retain "working memory" information across trials, and other perhaps longer periods of time, the memory store conceptualized by Honig (1978) must be characterized by an ability to survive distraction. The second difference between these two concepts of "working memory" concerns the degree of control exerted over each store by the subject. Honig's concept of "working memory" requires a high degree of conscious control to be maintained by the subject, both in retaining information and in resetting the information when a change is appropriate. In contrast, "working memory"

for cognitive psychologists does not imply this high degree of control. Given that the two definitions of working memory possess some important and incompatible differences, a good case may be made for suggesting that a replacement term be found for Honig's concept of "working mmemory" in the interest of clarity. However, for the purposes of this paper, the term "working memory" will be used in the same sense that Olton and Honig have defined it.

Using this approach, Olton et al (1979) then investigated the effect of hippocampal lesions on a number of different tasks, and concluded that damage to the hippocampus impaired the working memory processes required to perform tasks with working memory procedures. One important fact concerning the experiments described by Olton et al (1979) is that they employ preoperative training, and hence are designed to investigate the effects of hippocampal damage on performance rather than acquisition, in order to test an hypothesis proposing a memory function of the hippocampus. Since the working memory hypothesis was originally formulated to provide an alternative explanation for some data not readily handled by the spatial map hypothesis, Olton et al (1979) include the following in their introduction:

> "The purpose of this paper is to compare the usefulness of these two general approaches in describing the behavioral changes following hippocampal system damage in rats in a series of experiments using a radial-arm maze. The initial

> studies showed that in a test of spatial memory, rats with hippocampal system damage were severely impaired. Subsequent studies systematically varied the spatial and the memory characteristics of the task. They demonstrate that the critical variable responsible for hippocampal involvement was the memory requirement of the task and not its spatial nature. We see these data as compatible with memory interpretations of hippocampal functions, but not spatial ones. "
> (p. 313)

Before describing in greater detail some of the experiments referred to in this quotation, it is appropriate to discuss some of the points made in the passage. To begin with Olton et al (1979) continually distinguish the spatial nature of the task from its memory requirements. It would appear that this tendency is premature at best, for it is difficult to see how an animal's retaining information about its environment in the form of a neural representation of a map could be called anything other than memory function, albeit a highly specialized one. While this may be a minor point as far as Olton et al (1979) are concerned, it is worthwhile to clarify it for the present purposes, since both the spatial map and the working memory hypotheses are characterized as hypotheses which suggest a memory function for the hippocampus.

Olton et al (1980) describe a series of experiments which were run to test the implications of the working memory hypothesis. These were systematically organized into categories depending on whether spatial maps and/or working

memory could be used in the tasks employed by the individual studies. Olton et al (1980) presented a four cell matrix, each cell corresponding to one of the possible combinations of memory requirements (working or reference) and mapping requirements (mapping permitted or not permitted).

The study representative of the first category involves a spatial component (i.e., the maze is in a fixed location with fixed extramaze cues present around the testing room) and a working memory component, a win-shift strategy. Becker, Walker, Olton, & O'Connell, (1978) reported that hippocampal lesions produced an enduring deficit in this task using an eight-arm radial maze, while Olton and Werz (1978) reported a similar deficit using a 17-arm radial maze. These results are interesting but unfortunately the working memory component and the spatial component of the task are confounded. Consequently they can not be regarded as valid tests of the working memory-spatial map hypothesis distinction.

The crucial design to test between the spatial map hypothesis and the working memory hypothesis requires a comparison between a working memory task which includes a spatial component, and one which does not. Such a task was used by Olton and Feustle (1979), involving a four arm radial maze in which intramaze cues were made salient by increasing the height of the arm walls and including visual

and tactile stimuli. Extramaze cues were minimized by covering the tops of the arms with cheesecloth and reducing the room illumination. Use of a spatial strategy was further excluded by randomly switching each arm between trials such that the arms did not maintain a constant topographical relationship with each other over trials. Using this task they found that normal subjects took longer to learn the intramaze cue task than the extramaze cue task, and that following hippocampal lesions performance dropped to near chance levels. Thus with no spatial component present, subjects were impaired on a cue-based version of the radial arm task.

Olton et al (1979) then describe a study in which the apparatus is in a fixed location, thus allowing a place strategy to be used by the subjects. However in this case the task contained distinct working memory and reference memory components (Olton & Papas, 1979). A 17-arm radial maze was used, and eight of the arms were always baited at the beginning of every trial, while the remaining arms were never baited throughout the course of the experiment. The working memory component of this task was identified by Olton and Papas (1979) as being the fact that the same eight arms were always baited at the start of a trial, hence the subject needed to remember the identity and order of the arms entered on any one trial. The reference memory component consisted of the fact that the remaining arms

were never baited, and hence should always be avoided. Two patterns of baited arms were used; a mixed pattern and an adjacent pattern in which all baited arms were grouped together. Entries to unbaited arms were scored as reference memory errors, while reentries to arms from which the subject had, on that same trial, previously removed the bait were scored as working memory errors. Subjects were trained preoperatively until they learned to ignore the unbaited arms and showed few reentry errors. Following surgery, performance on both the reference and working memory components was equally impaired for rats with fimbria-fornix lesions. After about thirty tests performance on the reference memory component had returned to pre-lesion levels, while the performance of the working memory component remained at chance levels. These findings are taken as support for the working memory hypothesis.

Some comment is appropriate here. To begin with the finding that fimbria-fornix lesions produced equal impairents in reference and working memory procedure performance suggests that subjects tended to enter arms randomly immediately post lesion. Surely a more parsimonious interpretation for this aspect of the data is that the hippocampal damage could have rendered the subjects unable to distinguish the different arms by impairing their ability to navigate on the maze. It should be remembered that the maze employed had identical arms

which were distinguishable primarily on the basis of the extramaze room cues available. In this task the use of a place strategy to locate specific arms is required, hence the finding of equal impairments for baited and unbaited arm entries is exactly what the spatial map hypothesis would predict. The later data, then, is the only data which may be argued to exclusively support the working memory hypothesis.

The studies discussed so far pertain to three of the four cells in the matrix Olton et al presented. The remaining cell involves an experiment with working memory components absent and mapping permitted in one task but not in a second. The study (Becker, Olton, Anderson, & Margolies, 1979) reviewed by Olton et al is of great importance to the Experiment Three, since it represents a complementary situation to the conditions employed in the present experiment. Before discussing the details of the Becker et al study, it is important to deal with its implications and the predictions arising from its design. Since it does not involve working memory elements, the working memory hypothesis would predict no impairment following interference with the hippocampus. The spatial map hypothesis predicts a dissociation of the effect of hippocampal lesions on performance, since one task requires spatial mapping while the second does not permit it. Thus it can be seen that the results of such a study are crucial

for the working memory hypothesis. Further, they allow a clear distinction between outcomes which support the working memory hypothesis and those which support the spatial map hypothesis. Finally, it is of great interest to contrast the results of the Becker et al study with those reported for Experiment Two to be described below.

The study in question involved a central square arena within an enclosure. The area outside the arena was called the runway and each wall of the arena contained three doors. The task was to enter the arena through the only open door available and approach one of the distinctive objects present which concealed food. In the condition which did not permit spatial mapping, the food was consistently associated with the identity of the objects, and not their location. To ensure no mapping was used the objects and the points of entry to the arena were randomly arranged. The condition permitting mapping involved an identical apparatus, and conditions, with the exception that the location of the objects was constant over trials. The results of this ingenious experiment were somewhat troublesome for the working memory hypothesis, in that the dissociation following lesions which was predicted by the spatial map hypothesis was confirmed. The rats employing spatial strategies to solve the task were severely impaired while those which had to discriminate the identity of the object were unimpaired by the lesion. Thus this experiment,

while necessary from the point of view of systematically investigating the implications of the working memory hypothesis, actually provides strong support for the rival spatial map hypothesis. Lastly, the Becker et al study will be seen to be quite similar, in many respects, to Experiment Two, particularly the fact that both designs invite predictions, where the spatial map hypothesis is concerned, of a dissociation of stimulation effects on use of place versus guidance strategies. Experiment Two differs, though, in that it invites the working memory hypothesis prediction that both groups will be impaired following stimulation since the design contains a strong working memory component. The remainder of this section will be devoted to a brief summary of the study after which Experiment Two is closely modelled, followed by a description of the design of Experiment Two.

The spatial map hypothesis and the working memory hypothesis of hippocampal function have engendered a large amount of controversy in the recent literature. As is apparent from the above discussion a major reason for this is that many of the earlier working memory studies employed spatial tasks, such as radial mazes, thus introducing a source of confounding. One of the purposes of Experiment Two is to eliminate this confounding by controlling the spatial and working memory components of the experimental tasks independently.

Although Olton et al (1979) concentrate on performance effects, there have been few studies which have examined the effect of hippocampal stimulation during performance of a working memory task. Recently, however, one interesting study has reported that low-level unilateral stimulation of the dentate granule cells in rats produces a marked retention impairment in a radial maze task (Collier, Miller, Travis & Routtenberg, 1982). Since their study provides the starting point for Experiment Two, it warrants a brief description.

Rats were first trained in an eight-arm radial maze on a standard task involving a "win-shift" strategy. In other words, errors consisted of reentering arms already visited on a given trial. Rats were given one daily trial to a criterion of 7-8 correct entries per trial over five days. Chronic unilateral dentate gyrus monopolar stimulating electrodes were then implanted using electrophysiological placement techniques. Training was then given on a task involving delayed matching to sample in the radial maze. This task involved a "win-stay" strategy which meant that S's had to learn to locate the only baited arm on trial one and return to that arm only on trial two. S's were given two trials per day for this task. Following mastery of this task S's were shifted to a version of this delayed match to sample task which involved five daily trials. As before, S's discovered the baited arm on trial one and were

required to return to that arm only on subsequent trials. Trials two and three were to show that the rats had indeed mastered the task. All trials were separated by a one minute intertrial interval (ITI), and for the first 30 seconds of the ITI between trials three and four unilateral stimulation of the dentate granule cells was given (60 Hz sine wave, 10uA peak intensity). The effect of this stimulation was to produce a marked impairment in performance on trials four and five. On trial four this impairment consisted of increased errors for choice accuracy (failure to find the baited arm) and choice repetition (reentry into arms already entered on that trial), while for trial five only choice accuracy errors persisted. Trial five performance overall was better than trial four but S's were still significantly impaired over controls and their own earlier performance on trials two and three. In total three stimulation days were given, over six days of trials, with the result that no change in the degree of impairment was seen over accumulating amounts of stimulation.

Collier et al, 1982 concluded that these results revealed a distinct retrograde amnesia (RA) and anterograde amnesia (AA) effect. They suggested that errors in choice accuracy reflected a RA effect since in this case the subject could not recall information about the correct baited arm learned prior to stimulation. In contrast, an AA

effect was revealed by errors in choice repetition since in this case, S's were failing to remember information learned after the stimulation. It was the persistence of the choice accuracy effect which led to the conclusion that working memory (which they equated with choice accuracy) was impaired by the stimulation.

This conclusion is open to question on a number of grounds. The prime problem with their conclusion concerns the fact that, as with many of the other studies mentioned in this discussion, there was a strong spatial component in the task which is confounded with the working memory factor. Stimulation-induced impairments in the spatial capacities of the S's could be equally expected to produce random entries to arms, as in the case of the Olton & Papas study (1979). Thus the interpretation that these error patterns reflect impairments of two different memory capacities of the subject, may be replaced by a more parsimonious explanation based on impaired spatial abilities of the subject as a result of the hippocampal stimulation. Subjects could be aware of the rewarded arm and of arms already entered on a trial, yet not be able to locate them due to pure spatial impairments. This explanation is even more feasible when it is realized that the apparatus and room cues alike were in fixed locations, a condition which encourages use of place strategies.

A second problem concerns the definition of working

memory employed. In this design there appear to be two areas in which working memory may be said to be required. Subjects must forget the relevant arm from the previous day on trial one of a given day, and once they discover the current relevant arm on trial one they must remember it for the rest of the day. This all comes under the heading of choice accuracy, to use the authors' definition. However, within this second task, there is a working memory component with an even more restricted time frame, namely the subject may make a reentry error once a choice accuracy error has occurred. This is referred to as a choice repetition error. The results indicate that choice accuracy errors are longer lasting, yet it must be concluded that both types of errors reflect working memory impairments. A major problem arises from these data in that the working memory hypothesis makes no prediction concerning different time courses of these separate working memory effects. In addition, the working memory component of this experiment which is most directly comparable to the Olton et al (1979) definition is that of choice repetition within a trial, that is, reentry errors, which, in the Collier et al (1982) study, showed relatively quick recovery following stimulation. In contrast the enduring deficit in the Collier et al (1982) study was the choice accuracy component, an element which was operative across trials in the sense that the correct arm had to be remembered within

a day.

Since the Collier et al (1982) experiment was open to several interpretations it was decided to partially replicate the study, using the Morris water task as the experimental task, since the task may be solved using either spatial or non-spatial strategies, as demonstrated by Experiment One.

Design

This experiment was designed to test the effect of stimulation on performance of the Morris water task which involved a working memory component. As before, the design involved two groups given acquisition training on a task requiring either a taxon strategy or a place strategy. The MAP group was trained to ignore the cues suspended over the tank, and to rely on the fixed room cues in order to locate the platform. The CUE group was trained to associate a cue with the platform, and ignore the fixed room cues, since the location of the cue and platform combination varied randomly over trials. It should be noted that this design is very similar to that used in the previous experiments.

A second element in Experiment Two was the presence of a working memory component within the task for each group. As in the Collier et al (1982) study, the target memory item, in this case the location of the platform, rather than a baited arm, remained constant over all the trials in a single day, but changed over days. Thus the task employed

in this experiment was essentially a water maze equivalent of the Collier et al (1982) radial maze 'win-stay' task, one difference being that only four potential target items were present instead of the eight arms of the radial maze.

Following acquisition of this task by both groups, dentate gyrus stimulation was then given to half of each group. The predictions concerning the results for each group are clear-cut for the hypotheses being tested in this experiment. The working memory hypothesis predictions will be described first. Since both groups were required to perform a task involving a working memory component in the sense that Collier et al define it, disruption of the hippocampus by electrical stimulation would be expected to produce a marked impairment in both groups. In contrast, the spatial map hypothesis predicts that only the MAP group subjects would be disrupted by the stimulation, since they are the only ones which required an intact mapping ability to solve the task. Experiment One has shown that the MAP subjects were highly disrupted by the stimulation, hence it is reasonable to expect a similar disruption in this case. Thus the crucial results for this experiment were those for the CUE group, since this group must show some impairment if the working memory hypothesis is to be supported.

Following the first day of stimulation testing, it was decided to manipulate the presence or absence of the suspended cues for the MAP groups, and to counterbalance

the these conditions for groups M1 and M2. In other words the order in which a subject received stimulation and exposure to the tank with no suspended cues was counterbalanced. The reason for this was to discover whether the presence or absence of suspended cues had any affect on the reaction of the MAP subjects to the initial presentation of stimulation. The resulting design is illustrated in Table 3. It can be seen the presence of cues factor and the presence of stimulation factor were treated as within subject factors, while the strategy factor and the group factor were between subject factors. Since only MAP subjects had the cues removed from the tank Table 3 only shows one between subject factor, namely groups. It should be realized that a between groups factor of strategy with two levels, CUE and MAP, was also present. With this design any effect due to the presence or absence of cues would show up as a main effect, while the effect of the order in which the subjects were given stimulation and the suspended cues would appear in the various interactions involving the 'presence of cues' and the stimulation factors.

Table 3. Design For Treatment Of MAP Subjects Showing Counterbalancing of Suspended Cue Factor And Stimulation Factor Over Four Days Of Testing.

The table shows that the suspended cues factor and the stimulation factor were treated as within subject variables. The number in each cell represents the actual day on which the conditions for that cell were fulfilled.

		CUE		NO CUE	
		STIM	NO STIM	STIM	NO STIM
	MAP	1	4	2	3
MAP					
	MAPCON	4	1	3	2

Method

Subjects

The subjects for Experiment Two consisted of 40 male Long-Evans hooded rats, obtained from Charles River in Quebec. Subjects ranged from 300 to 400 grams at the time of surgery. All subjects were housed together in a temperature-controlled room on a 24 hour continuous light cycle, and given ad libitum food and water throughout the course of the experiment.

Before acquisition training all subjects received identical bilateral monopolar electrode implants in the stratum moleculare of the dentate gyrus. After seven to ten days for recovery acquisition trials began. Prior to training subjects were randomly allocated to two groups of twenty subjects each, referred to as a MAP group and a CUE group, as in the previous experiments.

During training, a total of three subjects lost their electrode assemblies and had to be discarded.

Surgical Procedures

Surgical procedures for this experiment closely followed those for the previous experiments. In this case only a single electrode was implanted in each dentate gyrus at the level of the stratum moleculare. The same stereotaxic coordinates with respect to bregma (AP, -3.5 mm; L, 2.0mm), and the same technique of electrophysiological implantation was used.

Following surgery each subject was replaced in the home cage and allowed about a week to recover.

Preparation of Electrodes

Each electrode consisted of a single length (approx. 10 - 12 mm) of fine Teflon coated stainless steel wire (0.0092 mm with Trimethyl insulation, commercially available from Johnson Mathey Metal Ltd.). The insulation was removed from the tip of the electrode for a distance of 0.5 mm and from the other end for a distance of about 4 mm, to allow a site for connection to the stimulating leads. Electrodes were held vertical in an alligator clip attached to the stereotaxic electrode holder.

Apparatus

All apparati used in Experiment Two were identical to those described for the previous experiment. This includes the tank itself, the video equipment, and the stimulating apparati.

Three cues in addition to the black cube were suspended over the tank during trials:

1. A white golf ball with a vertical and horizontal black stripe painted on it.
2. A black and white checkered cone made from an inverted funnel three inches in diameter.
3. A white styrofoam 2.5 inch ball into which a number of thin sticks, each with a smaller colored sphere on the end distal to the ball,

were randomly inserted. This cue will be referred to as the 'star'.

Thus the cues used in this experiment consisted of a ball, a cone, a cube and a star, each of which was suspended over the tank during trials.

Procedure

The procedures followed for each trial, training and stimulation, were identical to those described for the previous experiments. The only difference in the present experiment concerned the protocol involving the four suspended cues and the four potential platform locations. Training consisted of gradually introducing more cues and platform locations into the experiment until subjects showed relatively direct swim paths to the current relevant cue. During training care was taken to ensure that the CUE group was given equal exposure to all four cues by the time stimulation was given, and that each cue was the relevant cue for an equal number of trials. The same applied to the MAP group, except that in their case care was taken to ensure that each platform location was relevant for an equal number of trials. The protocol followed to achieve this state of training is given below. To simplify description the references to the cues apply to both groups. That is, the MAP group experienced the same cue configurations as the CUE group on each trial. The difference between the groups consisted of the fact that

the platform location remained constant for the MAP group regardless of the cue positions. The platform location moved with the relevant cue for that day for the CUE group. All cues used on a given day were used for all trials on that day. One final difference from previously described procedures was that on each trial all subjects from both groups were introduced into the tank from the same starting location, while starting locations varied over trials so that each location was used once in every four trials. Also subjects were run in groups of nine or ten during acquisition trials. For stimulation trials subjects were run through all eight trials in a row to obtain a better picture of any recovery effects which might have occurred.

Days One To Four. Only four trials were given on each of these days, since, as before, subjects tended to swim long distances during initial trials and it was desirable to avoid fatigue effects. On each day only one platform location and one cue was used.

For the MAP group only two of the four locations were used over the first four days, on alternate days. For the cue group only two of the cues were exposed, the cube and the ball, again on alternate days.

Day Five. On day five the third cue, the cone, and the third platform location were introduced. Eight trials were given on day five, and for the remainder of the experiment. The reader is reminded that on each trial to this point

only one cue had been present on each trial.

Day Six. On day six the fourth cue, the star, and fourth platform location was introduced. In addition this day was the first day in which there were two cues suspended over the pool on each trial. The star was the relevant cue for this day, resulting in each cue being associated with the platform a total of eight times by the end of day six. Similarly each platform location was exposed an equal number of times to the map group.

Day Seven. This was the second and last day in which two cues were present over the tank.

Days Eight To Ten. During this period three cues were present on each trial. By the end of day ten each cue had signalled the platform for a total of 16 trials, and each platform location for the MAP group had been used 16 times. Also each cue had been exposed for a total of 32 trials.

Days Eleven To Fourteen. This series of days constituted a second complete set of trials in which three cues were present. Thus by day fourteen all cues had been relevant for a total of 24 trials, and all platform positions had been used 24 times.

Days Fifteen To Twenty-Two. This period consisted of two four day sets of trials in which all four cues were now present over the tank. At the end of day twenty-two all subjects had received 160 trials during which all cues had been exposed an equal number of times.

Day Twenty-Three. Following day twenty-two the MAP group was divided into groups M1 and M2, and the CUE group was divided into groups C1 and C2, in a similar manner to that used in Experiment One. On day twenty-three groups M1 and C1 received unilateral dentate gyrus stimulation (18 microamps peak amplitude at 60 Hz) for the thirty seconds during the ITI between trials three and four. Following this the subject was immediately placed in the tank such that it had to swim across the tank to reach the platform. Once on the platform the subject was removed and the cue arrangement was changed appropriately. The subject was then placed back into the tank, again at an entry point across from the platform. This was repeated until eight trials had been run. The remaining halves of each group were treated identically with the exception that they received no stimulation.

Days Twenty-Four & Twenty-Five. Following the first day of stimulation it was discovered that little effect seemed to have occurred as a result of the stimulation, which was inadvertently of greater intensity than the 10 microamp amplitude used in Experiments One and Two. Consequently it was decided to give the subjects two days rest to recover from any effects of the stimulation, followed by a series of four bilateral stimulation days at the previously used stimulation parameters of 10 microamps and 60 Hz.

Day Twenty-Six. Before stimulating the subjects again a

day of training was given to counteract any decrease in performance level due to the previous two days rest.

Day Twenty-Seven. On this day groups M1 and C1 were stimulated between trials three and four. The procedures followed for these stimulation trials are identical to those described above.

Days Twenty-Eight & Twenty-Nine. Due to the nature of the effect the stimulation was apparently having, it was decided to train the groups for two additional days. During this time the cues were removed from the tank for the MAP groups, while conditions for the CUE groups remained unchanged.

Day Thirty. Groups M1 and C1 were stimulated on this day. For the MAP subjects the cues were not present on these trials.

Day Thirty-One. An additional day of training was given at this point to allow recovery from the effects of the previous day's stimulation.

Day Thirty-Two. On this day groups M2 and C2 were stimulated, while the remaining groups acted as controls. Once again cues were not present for group M2.

Day Thirty-Three. This day represented the final day of testing in this experiment. Once again groups M2 and C2 were stimulated, while the remainder acted as controls. For this day the cues were replaced into the tank for the M2 subjects.

Some elaboration on the manipulation of cues for groups M1 and M2 over these last four stimulation trials is appropriate. The results of the stimulation on group M1 made it necessary to investigate the effects that presence of the cues was having on the MAP subjects. Consequently the cues were removed and the stimulation repeated. In order to counterbalance the conditions under which the initial experimental groups (M1 & C1) were stimulated, it was necessary to stimulate the previous control groups (M2 & C2) first with the cues removed, and then with the cues present.

To briefly summarize the procedure, rats were trained to use either a place or cue strategy on a version of the MWT which included a working memory component, namely that CUE subjects had to return to the same hanging cue for all trials on a given day, and MAP subjects did the same for the particular relevant location for that day. Both groups were trained by a method of successive approximations until they had become equally familar with all four platform locations and were at asymptote. Thus each subject was required to remember the platform location for each trial of a day, and then reset this memory with the information obtained on the following day.

Results

Histological Results

The procedures employed for perfusing, fixing, embedding, sectioning, and mounting the brains of the subjects of Experiment Two were identical to those described for Experiment One. Once again all placements were found to be extremely accurate, located on the hilus of the dentate gyrus, or just below in the molecular layer per se. Figure B in Appendix 2 provides some representative sections from the Experiment Three subjects, showing the tip of the electrode along the hilus of the dentate gyrus. Note that, since only a single electrode was used, the electrode track is much narrower than in the Experiment One subjects. There were two subjects in which one electrode was not precisely located along the hilus, but instead was located either in the pyramidal layer, or below the molecular layer. However in both cases the contralateral electrode placement was accurate.

The rather high degree of accuracy concerning the electrode placements attests to the value of employing physiological recording techniques during implantation. The hilus of the dentate gyrus was found to be quite distinguishable on the basis of the characteristic bursting pattern which occurs when it is reached. This technique makes it relatively simple to align electrodes precisely along the hilus of the dentate gyrus.

One problem was found to be present in some of the sections obtained from the subjects. It appeared that in many cases a circular area of gliosis was present along the electrode track or at its tip. The last sections shown in Figure B in Appendix 2 show some examples of this gliosis. It is possible that these areas were due to infection. Although the electrodes were immersed in an alcoholic soap solution prior to being implanted it is possible that they may have come into contact with the non-sterile edge of the drill hole when being lowered into the skull, or possibly some other source of contamination was contacted prior to implantation.

Whatever the cause, this discovery necessitated some post hoc analyses of the data to determine the degree to which the presence of infection affected the acquisition performance of the subject. The analyses were carried out as follows. First the MAP and CUE groups were divided into three subgroups according to the extent of the infected area found in each. The acquisition data for each trial over the first four acquisition days (sixteen trials in all) was then analysed using an analysis of variance with strategy and extent of infection as between subject variables and days and trials within days as within subject variables. A significant strategy by infection interaction was found $F(2,23) = 4.41$, $p < .02$ and the interaction is shown in graph A of Figure 13. It can be seen that the

distance swum increases as the extent of the infected area increases. This finding suggests that the presence of infection produced some initial impairment of acquisition for the MAP group. To determine the longevity of this effect an identical analysis was performed using the data from days seven and eight of the acquisition phase. No significant interaction involving the infection factor was obtained from this analysis and the non-significant strategy by infection interaction from this part of the data is shown in graph B of Figure 13 for comparison. It can be seen that the differences have disappeared by day eight of the twenty-two acquisition days. This latter analysis suggests that the effect of the infection on acquisition had been eliminated well before the stimulation trials started. However, identical analyses were performed using the data from the first two bilateral stimulation days and the last two stimulation days. The non-significant strategy by infection interactions obtained from these two analyses are presented in Figure 14, where graph A is from the first two stimulation days and graph B is from the last two. In both cases, it can be seen that there is little difference among the the infection groups, and in fact the trend toward increased distance with increased area of infection is reversed in graph B. Once again no significant effects were found which involved the infection factor. Lastly, a similar analysis was done on a second

Figure 13. Graphs Of The Strategy By Infection Interaction For Days 1 To 4 And Days 7 And Eight Of The Acquisition Phase.

A - Graph of the significant strategy by infection interaction obtained from the first four acquisition days. Note that the MAP group is most affected by the extent of the infection, while the CUE group shows little change over all levels of infection.

B - Contrasting graph of the non-significant strategy by infection interaction obtained from data for days seven and eight. Note that the overall distance is much reduced from Graph A, and that no difference is present between MAP and CUE groups.

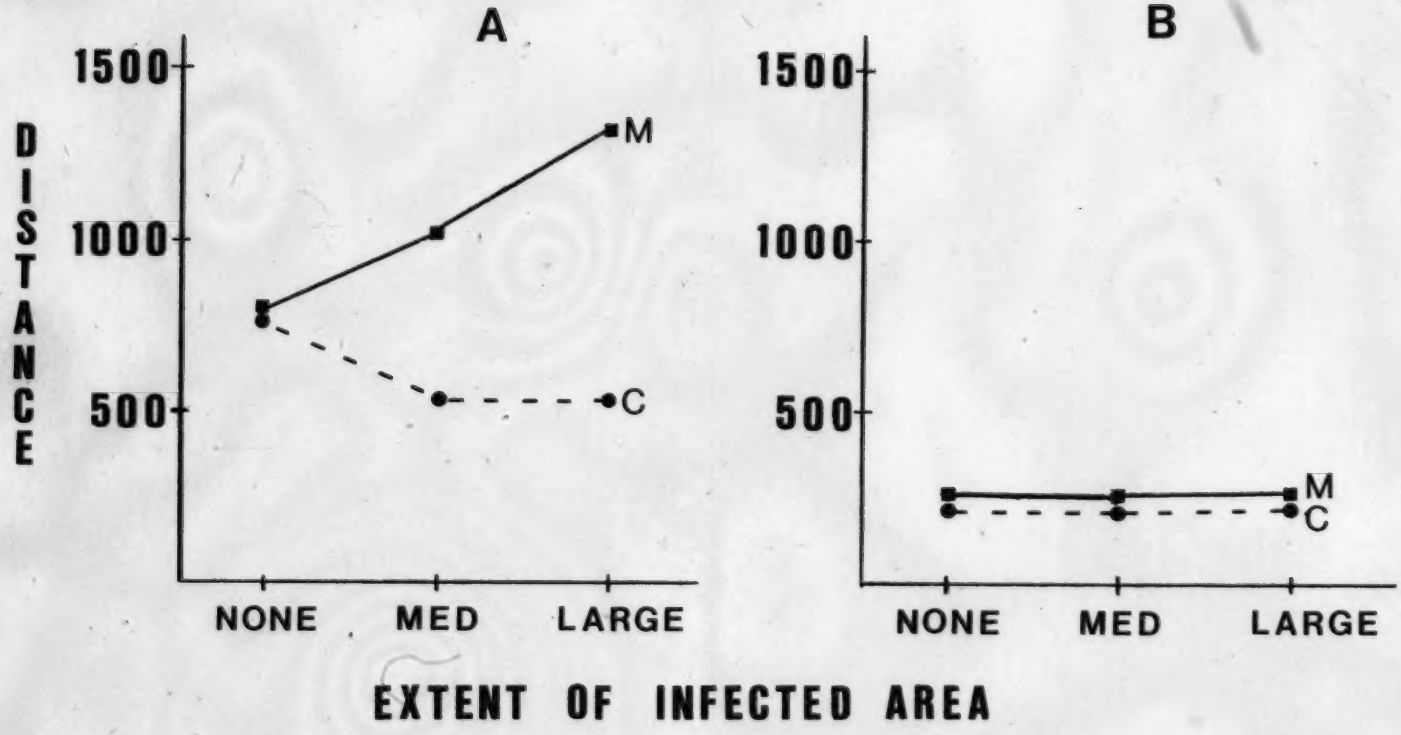
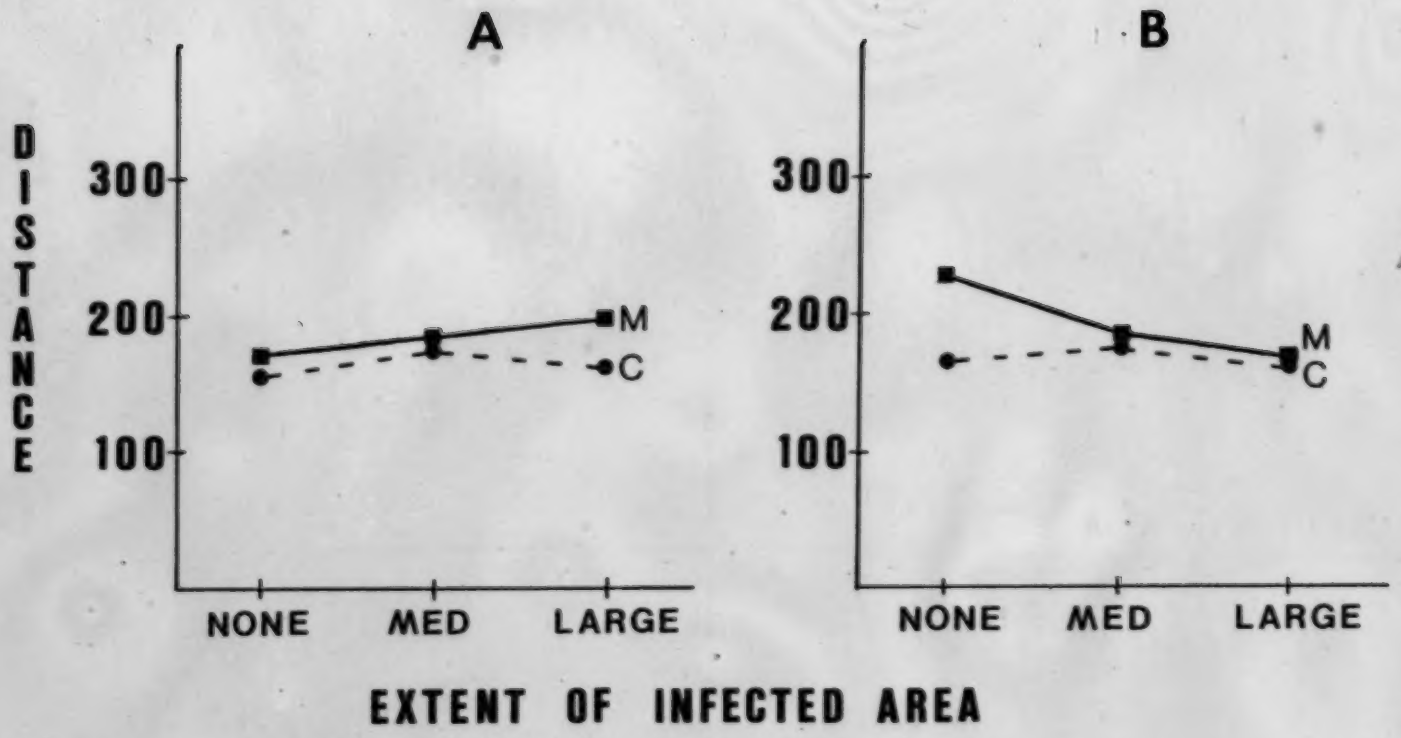

A
B
1500
1000
500
DISTANCE
M
C
NONE
MED
LARGE
EXTENT OF INFECTED AREA

Figure 14. Graphs Showing The Non-Significant Strategy By Infection Interactions Obtained From The First Two And Last Two Bilateral Stimulation Days.

A - Non-significant interaction from the first two stimulation days. Note that in both A and B the scale of the ordinate is different from Figure ?. Also note that the MAP group values are consistently higher in both A and B, due to the effects of stimulation.

B - Non- significant interaction from the last two stimulation days. Note that the curve for the MAP group indicates a reversal in the previously seen (Figure ?) trend for distance to increase with the size of the infected area.


A
B
DISTANCE
300
200
100
M
C
NONE
MED
LARGE
EXTENT OF INFECTED AREA

dependent variable which was of importance during the initial acquisition days. Although there was a strong main effect of strategy on the incidence of rearing, there were no effects involving the infection factor.

These findings, plus the finding that only the MAP subjects were affected, while both MAP and CUE subjects showed infected areas, suggest strongly that the impairments found during stimulation trials were due to the stimulation and not the presence of infection.

Finally it must be admitted, though, that the presence of the infection could have influenced the extent of the impairment produced by the stimulation. This issue will be discussed more fully in the Discussion section.

Consistency Of Stimulation Effect

Table 4 presents a summary of the consistency with which the stimulation produced impairment, in the MAP subjects in Experiment Two. The method of determining whether impairment has occurred was identical to that for Experiment One. It can be seen from the table that the overall average percent of trials showing impairment is again quite high for both groups. In fact in both experiments there was only one subject which did not show any impairment following stimulation. Thus this information, combined with the accurate placements found in these subjects, strongly invites the conclusion that low level stimulation of the dentate gyrus in rats severely

Table 4. Summary Of The Proportion Of Stimulation Trials In Experiment Two In Which M1 and M2 Subjects Showed Impairment.

GROUP	SUBJECT	STIMULATION DAY UNILAT 1	BILAT 1	BILAT 2	TOTAL
M1	1	YES	YES	YES	3
	2	YES	NO	NO	1
	4	YES	YES	YES	3
	5	NO	YES	YES	2
	6	YES	YES	YES	3
	7	YES	YES	YES	3
	8	YES	YES	YES	3
	9	YES	YES	YES	3
	10	YES	YES	YES	3
				TOTAL....24	
				PERCENT AVERAGE....89 %	
M2					
	1		YES	YES	2
	2		YES	YES	2
	3		YES	YES	2
	4		YES	YES	2
	6		YES	YES	2
	7		NO	NO	0
	8		YES	YES	2
	9		YES	YES	2
				TOTAL....14	
				PERCENT AVERAGE....88 %	

disrupts their ability to navigate in a familiar environment.

Behavioral Results

Rearing. Figure 15 shows the mean number of rears on each trial for the MAP and CUE groups for the first 12 trials of acquisition training. It can be seen that the MAP group reared consistently more over these trials, as graph B in Figure 4 shows for Experiment One. It is also worth noting that the maximum figure reached by the CUE group is five rears, while most remaining trials for this group show only three or four rears. An overall analysis of variance showed the main effect of strategy to be highly significant $F(1,24) = 15.45$, $p < .001$.

Swim Distances During Acquisition. Figure 16 shows the initial acquisition curve for both groups over the first four trial days (16 trials). The figure shows the mean distance swum by each group on each trial, with each day delineated by a vertical dotted line. The reader is reminded that conditions for these four days consisted of a single suspended cue being present for the CUE group, and one of two possible platform locations being used for the MAP group. The figure reveals an overall similarity of the curves, with the exception of the peak on the first trial of each day for the MAP group. Excluding these trials there was no significant main effect for strategy over these 16 trials.

Figure 15. Mean Number Of Rears Over The First Twelve Trials Of Experiment Two.

As was the case in Experiment One (see Figure 4) the MAP group reared consistently more during the early acquisition trials, and was still quite higher than the CUE group by trial 12.

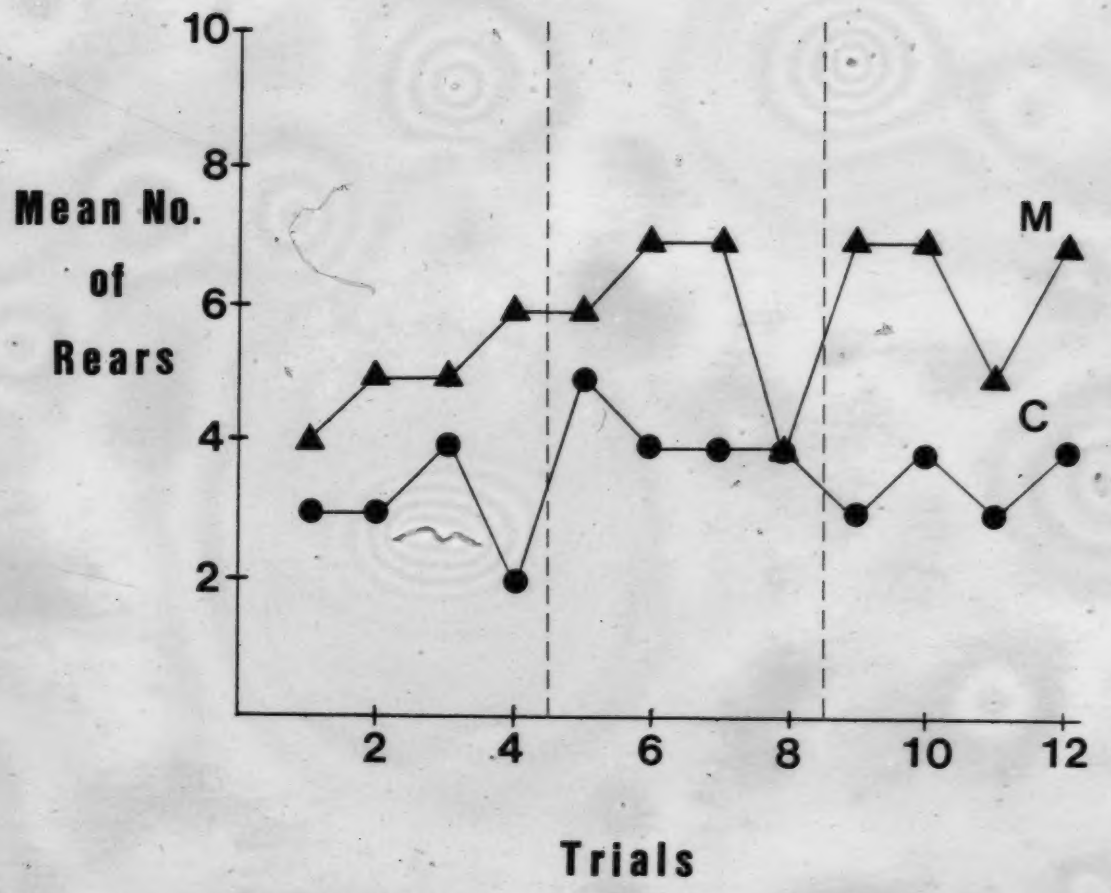
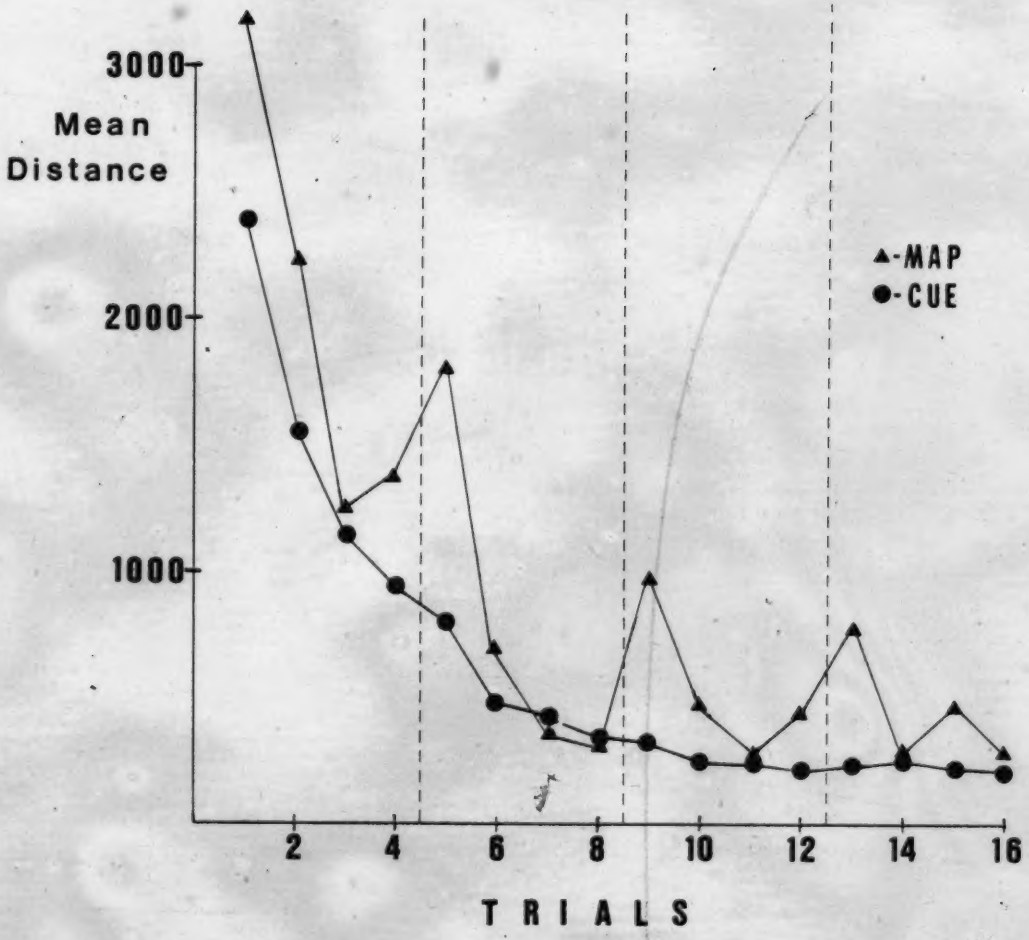

10
8
6
4
2
Mean No.
of
Rears
M
C
2
4
6
8
10
12
Trials

Figure 16. Mean Distance Swum By Both Groups Over The First Sixteen Acquisition Trials In Experiment Two.

It can be seen that the acquisition curves for both groups are quite similar, with the exception that the MAP group shows a distinct peak on the first trial of each day. This is due to the fact that the platform location was changed daily for this group, while the cue group had simply to follow the single suspended cue present on these four days. In spite of this, though, both groups show rapid acquisition of the task over these trials.

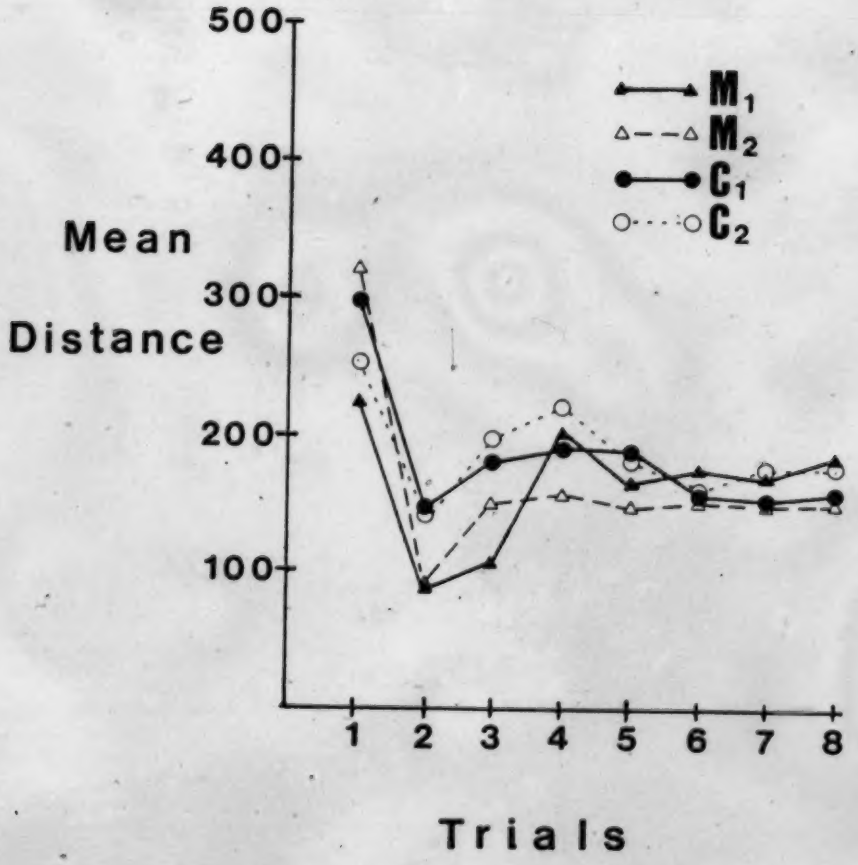

3000
Mean
Distance
2000
1000
▲-MAP
●-CUE
2
4
6
8
10
12
14
16
TRIALS

Unilateral Stimulation. Figure 17 shows the results of the unilateral stimulation given to groups M1 and M2 on trial 164. The graph shows the mean distance swum for all groups on each trial. Immediately following stimulation there appears to be an increase for group M1 (but this is non-significant) while group M2 remained consistently lower for all remaining trials. In contrast no such peak appears for the group C1, and it is consistently lower than group C2. The overall analysis of variance, however, revealed a main effect of strategy $F(1,32) = 12.02$ $p < .002$ but no overall main effect of stimulation for this day.

Bilateral Stimulation. A total of four days of bilateral stimulation were given, such that each group received stimulation for two consecutive days, and acted as a control for two consecutive days. The results of the stimulation were that both groups M1 and M2 were disrupted following stimulation, while the stimulation had no effect on groups C1 and C2. Figure 18 presents the significant three way stimulation by strategy by trials interaction obtained from these four days of stimulation trials $F(18, 162) = 1.69$, $p < .05$. The figure clearly shows that groups C1 and C2 (closed and open circles respectively) showed no effect of the single stimulation given prior to trial four. Not only is there no peak in the graph after this point, but both groups show remarkably similar mean distances throughout the graph. Also, if anything, they are slightly

Figure 17. Mean Distances Swum BY All Groups On The Unilateral Stimulation Day.

The graph shows that the swim distances were quite variable on this day for all groups. It is of interest to note that following stimulation (after trial 3) the curve for group M1 rises sharply and continues to be higher than group M2 throughout the remaining trials, suggesting a slight, but insignificant effect of the stimulation on this group. In contrast the curves of groups C1 and C2 cross each other several times, and show no sudden rise following trial three.


500
400
300
200
100
Mean
Distance
M1
M2
C1
C2
1
2
3
4
5
6
7
8
Trials

lower than the corresponding unstimulated CUE subjects in the right hand graph.

Independent t-tests were run to compare the groups on all trials following stimulation. It was found that stimulation of groups M1 and M2 resulted in a consistent disruption of performance, while the stimulation had no effect on groups C1 and C2. The results of these tests are shown by the distribution of symbols around the points in the graph showing stimulation results in Figure 18. The reader is reminded that interpretation of the symbols in Figure 18 follows the same scheme as for earlier Figures. However the appropriate control for each stimulated group in this case is the curve for that same group in the right hand graph. Thus the figure shows that group M2 following stimulation was significantly different from group M2 when it received no stimulation. As before, the comparisons between experimental (i.e., stimulated) groups are shown in the left hand graph. Thus group M2 was also significantly impaired with respect to group C2. Table 5 is a summary table of all these t-tests for trials four, five and eight. The following is a brief summary of the results for the bilateral stimulation days of Experiment Two. On trials four and five group M2 with stimulation swam significantly further than group M2 without stimulation and group C2 with stimulation. However group M2 with stimulation also swam further than group M1 with stimulation, a rather surprising

Figure 18. Graph Of The Significant Three-Way Interaction Obtained From The Four Days Of Bilateral Stimulation in Experiment Two.

The graph shows that only the M1 and M2 subjects were disrupted by the bilateral stimulation following trial three. In addition it can be seen that the effect is of longer duration in this case, since it is still quite high on trial eight. As expected all control groups show similar curves. Finally these results are based on data from four days of stimulation, two for each group.

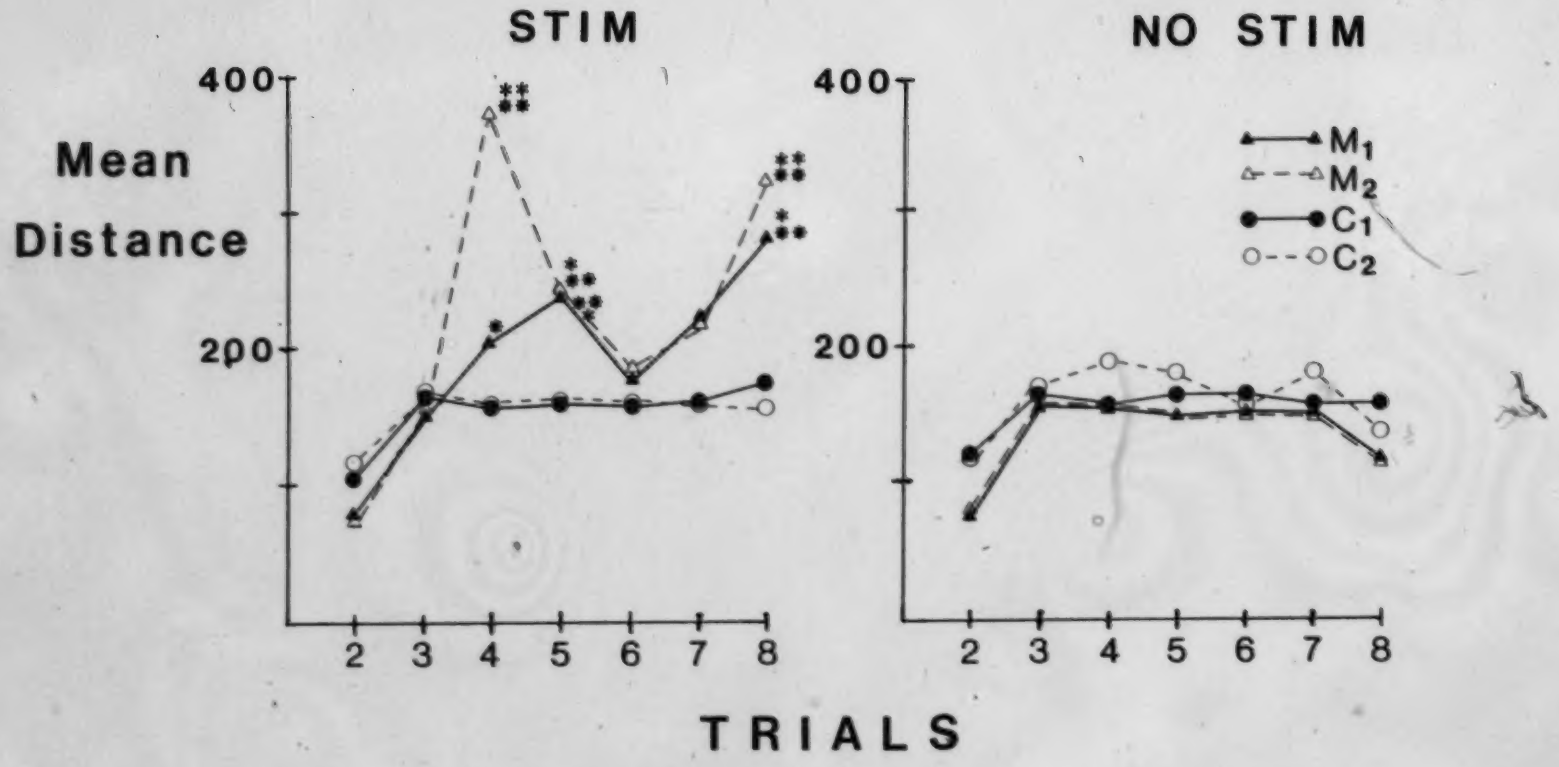

STIM
NO STIM
Mean
Distance
400
200
400
200
M1
M2
C1
C2
2 3 4 5 6 7 8
2 3 4 5 6 7 8
TRIALS

Table 5. Summary Table For Independent T-Tests Run On Selected Comparisons For Trials Four, Five And Eight.

The first part of the table concerns the within subject comparisons. Thus each stimulated group is tested against its own performance when non-stimulated. Thus the group names in each comparison are identical.

Within Subject Comparisons

Trial	Comparison	d.f.	T-Value	Probability
4	M1 vs M1	162	1.80	.10
4	M2 vs M2	162	7.22	.001
5	M1 vs M1	162	3.03	.01
5	M2 vs M2	162	2.83	.01
8	M1 vs M1	162	5.46	.001
8	M2 vs M2	162	6.89	.001

Between Subject Comparisons

4	M2 vs C2	162	4.94	.001
4	M2 vs C1	162	4.96	.001
4	M1 vs M2	162	3.88	.001
5	M1 vs C1	162	1.79	.10
5	M2 vs C2	162	1.72	.10
8	M1 vs C1	162	2.47	.02
8	M2 vs C2	162	3.81	.001

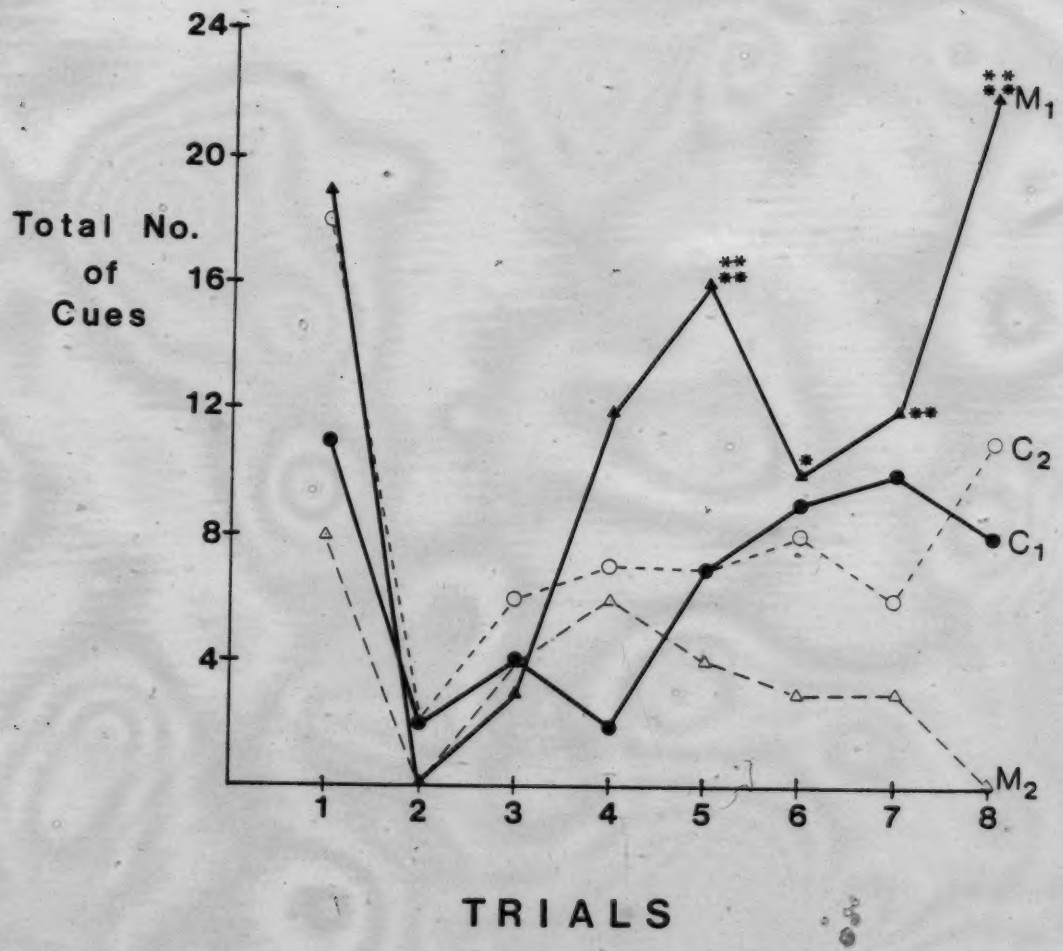
finding. Also group M1 with stimulation was not different from group M1 without stimulation on trial four, although the two groups were different on trial five. Finally groups M1 and M2 with stimulation were significantly different from their respective controls (M1 and M2 without stimulation) and corresponding experimental groups (C1 and C2 with stimulation) on trial eight.

The effect of bilateral stimulation seems to have been longer-lived than the unilateral stimulation used in Experiment One since a significant sharp rise on the final two daily trials appears in the graphs for groups M1 and M2. The corresponding non-stimulation trials for these groups show a consistently flat graph. It is interesting to compare the value on the final trial for the stimulated and non-stimulated M1 and M2 subjects. In contrast to the sharp rise which appears for the stimulated subjects, there is an actual decline for the non-stimulated subjects. (A similar pattern also appears in Figure 19 to be discussed below.)

Finally it should be emphasized that the overall analysis of variance showed no main effect for days, nor any significant interactions with days, as has been the case up to this point. However in this case the days factor was confounded with the presence or absence of suspended cues for groups M1 and M2 (see Table 3 to review the design for Experiment Two). Thus the absence of significant effects involving the days factor indicates

Figure 19. Total Number Of Cues Passed Under By All Groups On Day One Of Bilateral Stimulation.

The graph shows that following trial three there is a dramatic increase in the M1 curve which contrasts sharply with the M2 curve. The values on trial eight are particularly striking, since at this time the M1 group averaged over three cues while the M2 group did not pass under a single cue. Groups C1 and C2 are consistently intermediate to groups M1 and M2 throughout the day.


24
20
16
12
8
4
Total No. of Cues
M1
C2
C1
M2
1
2
3
4
5
6
7
8
TRIALS

that the effect of stimulation on the MAP subjects was constant whether or not suspended cues were present during stimulation days.

Figure 19 provides some further description of the stimulation effect seen in groups M1 and M2 following bilateral stimulation. This figure presents the total number of suspended cues under which each group swam on each trial of the first day of bilateral stimulation. The striking feature of this graph is the peak shown by the stimulated M1 subjects following trial four, which is contrasted by the corresponding decrease shown for the unstimulated M2 subjects. As mentioned above the difference is most dramatic on trial eight. The total value of 23 for group M1 indicates that on this trial stimulated M1 subjects typically visited three suspended cues before reaching the platform. Finally it is important to note that both groups C1 and C2 show no marked increase following trial three. In fact the stimulated C1 group actually decreases immediately following stimulation. This figure clearly shows that the effect of stimulation was confined to the stimulated MAP group, while both CUE groups appeared to be quite similar.

The overall analysis of variance for this dependent variable revealed a highly significant strategy by stimulation interaction $F(1,31) = 7.39$, $p < .01$. Independent t-tests were run on selected comparisons for

Table 6. Summary Table Of Selected Comparisons Made On The First Day Of Bilateral Stimulation For Groups M1 and C1.

Trial	Comparison	d.f.	T-Value	Probability
4	M1 vs M2	186	1.80	.10
4	M1 vs C1	186	1.80	.10
5	M1 vs M2	186	4.83	.001
5	M1 vs C1	186	2.72	.01
6	M1 vs M2	186	2.11	.05
6	M1 vs C1	186	0.54	N.S.
7	M1 vs M2	186	3.57	.001
7	M1 vs C1	186	1.87	.10
8	M1 vs M2	186	5.92	.001
8	M1 vs C1	186	3.57	.001

each trial following stimulation. Table 6 summarizes the results of these comparisons for trials four through eight. In general, the results showed that group M1 passed under significantly more cues than group M2 on trials five through eight. Group M1 also passed under more cues than group C1 on trials five and eight.

Verification of Strategy Employed By Cue Subjects

The fact that the pattern of suspended cues and potential platform locations was symmetrical within the tank allows the possibility that the cue subjects could have employed a simple orientation strategy to reach the platform on each trial. That is, instead of actually identifying the relevant cue for each day, and showing the desired win-stay working memory element of the task, the possibility exists that cue subjects may simply have maintained a constant distance between themselves and the tank wall, such that the swim path then passed under suspended cues in turn until the platform was reached. In this case, the presence of a working memory element in the task would be in doubt, as would the conclusion that the rats were actually learning the identity of the relevant cue on each new day.

This possibility made it necessary to perform some additional analyses on the data obtained for the cue subjects. Specifically the remaining videotape records for the cue subjects were reanalysed to obtain a raw error

score for each subject on each trial. Unfortunately the amount of videotape available during the experiment was limited, so that a number of earlier tapes had to be sacrificed to record later trials - thus the remaining trials on which this reanalysis was done included the last three days of acquisition and the remaining stimulation and recovery days to the end of the experiment. Each trial was scored as follows. On entry facing the wall each rat then turned so that it faced the cues. (The rats exhibited a remarkably consistent direction of turning over trials. Only two of 18 showed turns in both directions over the trials analysed.) The initial entry point and direction in which the rat turned was noted. An error was scored when the swim path of the rat passed underneath a suspended cue which did not signal the platform for that day. If the rat approached a cue but did not actually pass under it an error was not scored. Thus it was possible, and, in fact, a frequent occurrence, for rats to swim a curved path within the perimeter of the suspended cues before reaching the platform. An independent rater was asked to score all the trials for a single day for each cue subject using the same criteria. The interrater reliability coefficient thus obtained was quite high ($r = .91$).

When the error scoring had been completed, the analysis was performed in two ways. The first involved comparing the raw error score for each trial with the error

score which would be expected if the subject was employing the orientation strategy described above. The expected score was quite simple to calculate since the entry point, direction of initial turn, and location of the platform was known for each trial. If the rat simply visits the suspended cues in order until it reaches the one over the platform, the expected error score for that trial is the number of cues in the path before the platform. For example, when the platform is located in the south-east quadrant, if the rat is placed in the water facing the wall at the south entry point, and then turns to the right, the expected error score for that trial is three errors. (See Figures 20 and 21 for more examples of expected error scores.) The next step was to count the number of trials on which the observed error score differed from the expected, and to discover if the majority of these trials showed observed scores higher or lower than the expected. In general there were far more lower observed scores than higher. However the pattern of the suspended cues made it necessary to be slightly more selective in this approach. It was often observed that the turn made by the rat brought it directly underneath the closest suspended cue. This meant that for those trials where the expected score was zero little could be concluded with respect to the strategy the rat was employing. The same also applied to expected scores of one, since the path of the rat after making its

initial turn would take it under the first suspended cue automatically, regardless of whether it was using an orientation strategy, or did actually know the cue to approach. In the latter case the most direct route would be straight under the first cue. Thus it is the case that only those trials with an expected error score of two or three are of use in determining whether the subjects were using an orientation strategy. In this case lower observed scores would indicate that the rats were employing a win-stay strategy involving knowledge of the relevant cue for each trial. Thus the measure which is of interest is the percentage of those trials with expected scores of two or three errors on which the observed score was lower. The results were as follows. Group C-1 showed lower scores on 71 percent of these trials and group C-2 showed 67 percent. In view of the fact that very few of the trials should differ from the expected score if the subjects used an orientation hypothesis, these rather high percentages support the conclusion that the subjects had learned which cue to approach. A further point is that the above figures also include first daily trials. On these trials, when the subjects had not discovered which cue signalled the platform, use of an orientation hypothesis would be an optimum strategy, hence on these trials the observed and expected scores would be more likely to coincide. For this reason, the results were corrected by removing all the

Figure 20. Sample Swim Paths Produced By A C-1 Subject.

The figure illustrates the performance of a C-2 subject over an entire series of trials starting with the final three acquisition days. The solid line represents the swim path for that trial. The dotted line in the top eight trials are swim paths expected from rats employing an orientation strategy. Note the dramatic reduction in errors following the first trial.
The broken lines represent the pathways travelled following stimulation of the hippocampus. Note that stimulation has little disruptive effect.

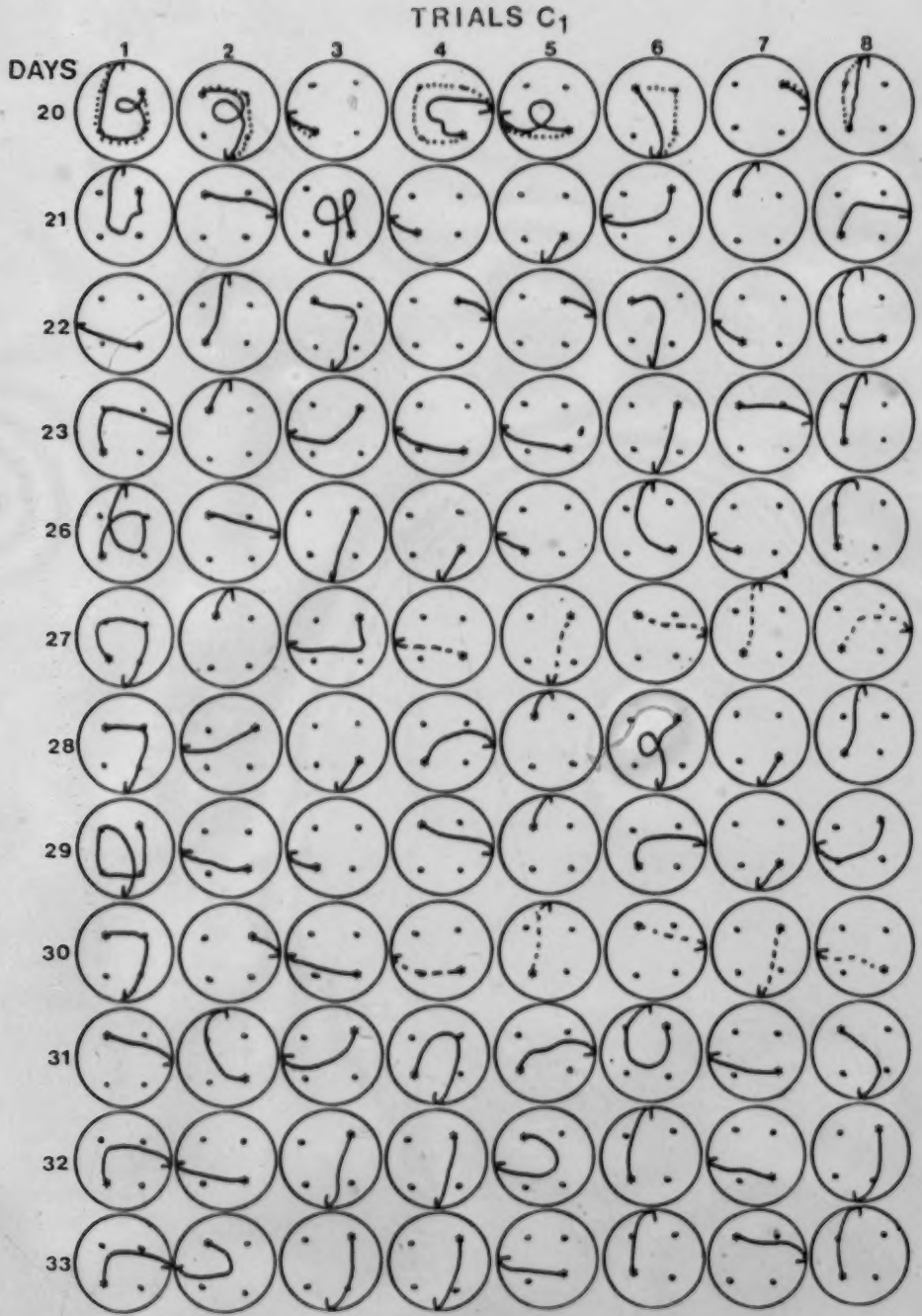
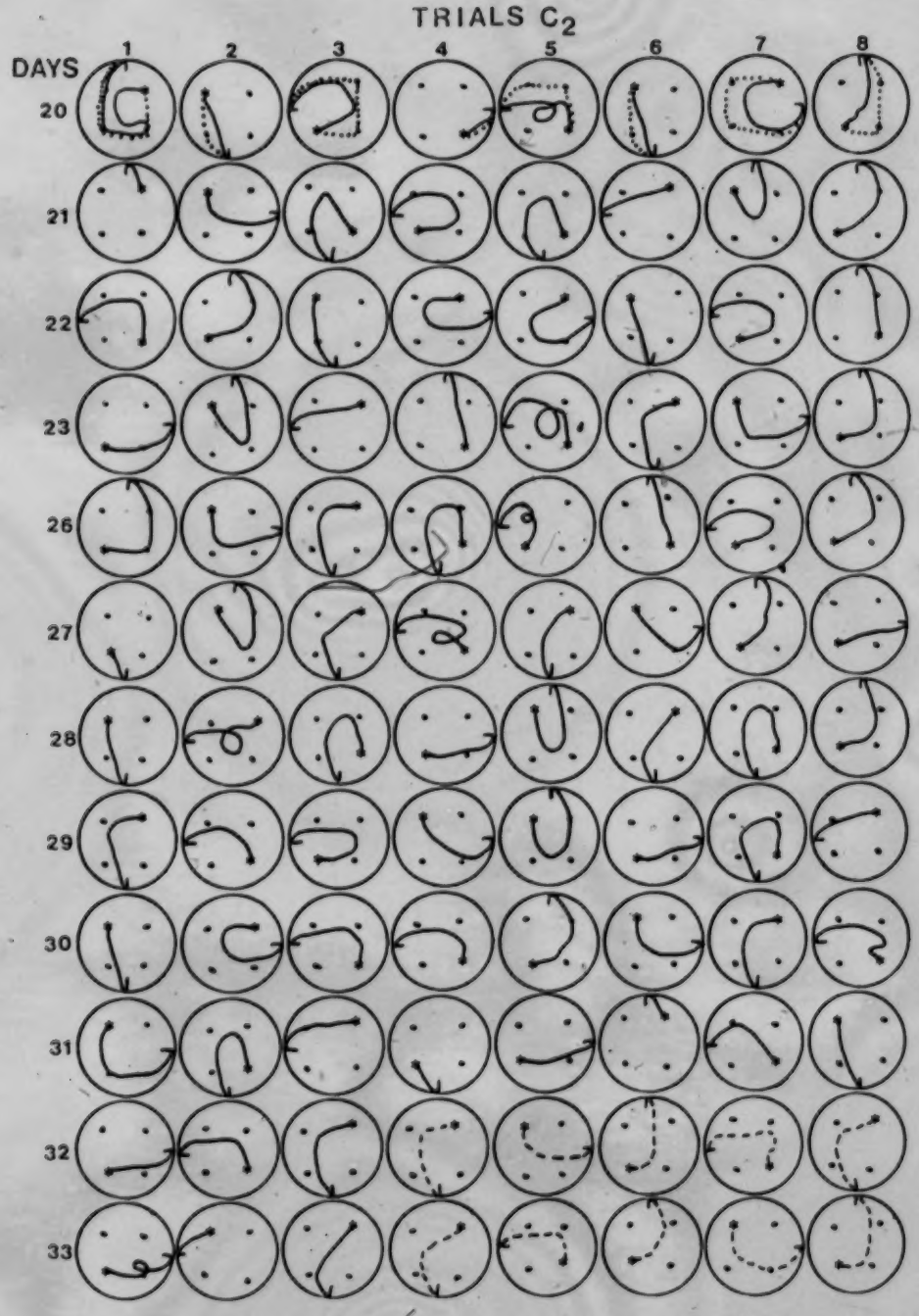

TRIALS C_1
DAYS
1
2
3
4
5
6
7
8
20
21
22
23
26
27
28
29
30
31
32
33

Figure 21. Sample Swim Paths Produced By A C-2 Subject.

The figure illustrates the performance of a typical C-1 subject over an entire series of trials starting with the final three acquisition days. The solid line represents the swim path for that trial. The dotted line in the top eight trials are swim paths expected from rats employing an orientation strategy. Note the dramatic reduction in errors following the first trial.
The broken lines represent the pathways travelled following stimulation of the hippocampus. Note that stimulation has little disruptive effect.


TRIALS C_2
DAYS
1
2
3
4
5
6
7
8
20
21
22
23
26
27
28
29
30
31
32
33

first trials from the analysis (of course, if a first trial happened to have a lower observed score anyway it was still eliminated along with that instance of a lower score). The corrected results were 80 percent for group C-1 and 76 percent for C-2. Thus it appears that on over three quarters of all the relevant trials the cue subjects did not show the expected number of errors with respect to the orientation hypothesis. Figures 20 and 21 illustrate the swim paths of a C-1 and C-2 subject respectively. It can be seen that in most cases the swim paths follow an orientation strategy on trial one. Following this, the number of errors declines such that the paths deviate markedly from the expected path (shown on the top set of trials in each figure).

The second approach centered on the observation that if rats were learning the identity of the relevant cue on the first trial and then displaying a win-stay strategy on subsequent trials, there should be a reliable decrease in raw errors following each first daily trial. Furthermore, the error scores for all the remaining trials should be consistently low. Thus separate analyses of variance were run on the error scores for acquisition days, C-1 stimulation days, C-2 stimulation days, and recovery days. In order to eliminate variability due to comparing different length trials (i.e., long versus short with respect to the entry point and platform location) only

DAYS	FACTOR	D.F.	F-RATIO	SIGNIFICANCE LEVEL(P<)
ACQUISITION (SHORT)	TRIALS	3,51	13.06	.011
	P. C.	1,17	18.14	.00053
ACQUISITION (LONG)	TRIALS	3,51	8.66	.008
	P. C.	1,17	14.85	.001
STIMULATION (GROUP C-1)	TRIALS	6,42	8.21	.007
	P. C.	1,7	17.79	.0004
STIMULATION (GROUP C-2)	TRIALS	7,35	4.07	.023
	P. C.	1,5	7.86	.038
RECOVERY	TRIALS	3,39	4.84	.015
	P. C.	1,13	19.44	.0007

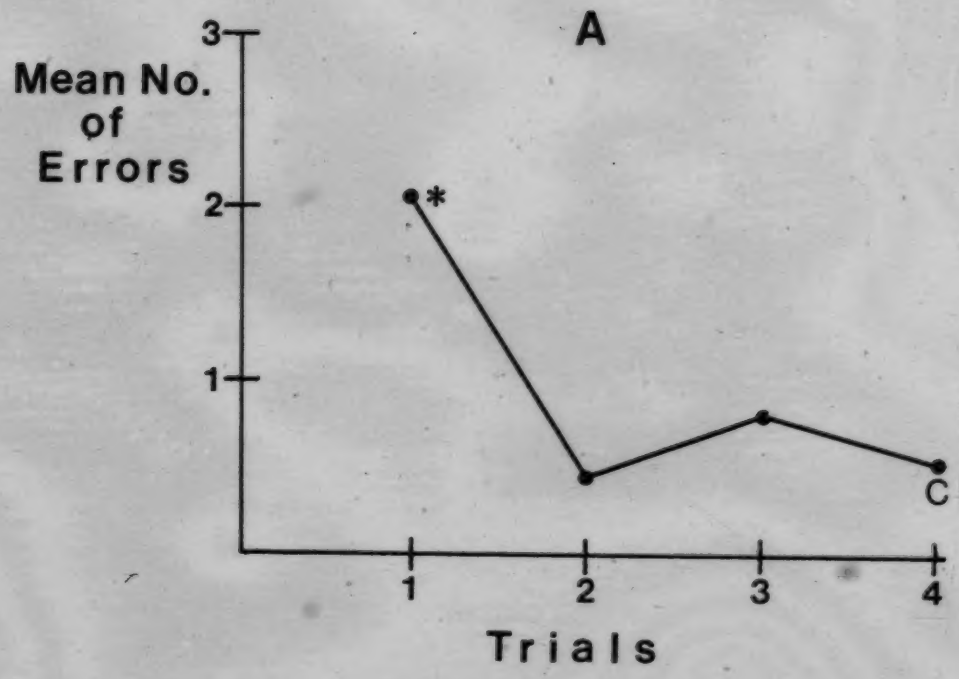
P. C. = PLANNED COMPARISON

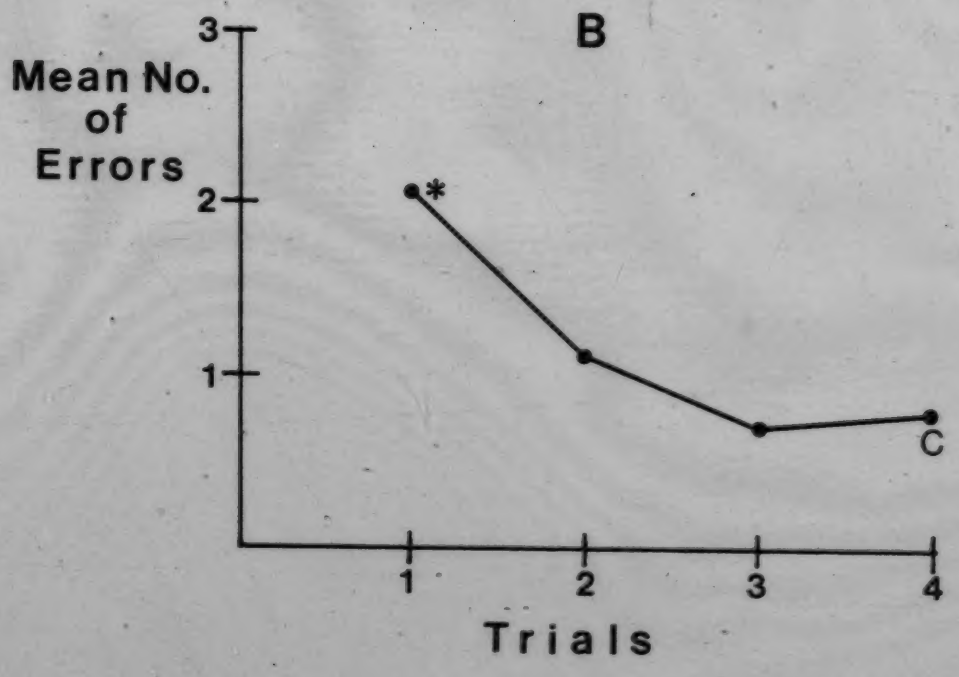
Table 7. Summary of Anova Results For Raw Error Score Analyses.

Figure 22. Graphs Showing Mean Errors For All Cue Subjects Trials During Acquisition.

A. The graph shows mean error performance on short trials collapsed over two days Note the sharp decline in errors following trial one.

B. The graph shows mean error performance for long trials during the final day of acquisition. A similar decrease following trial one is seen in this graph.



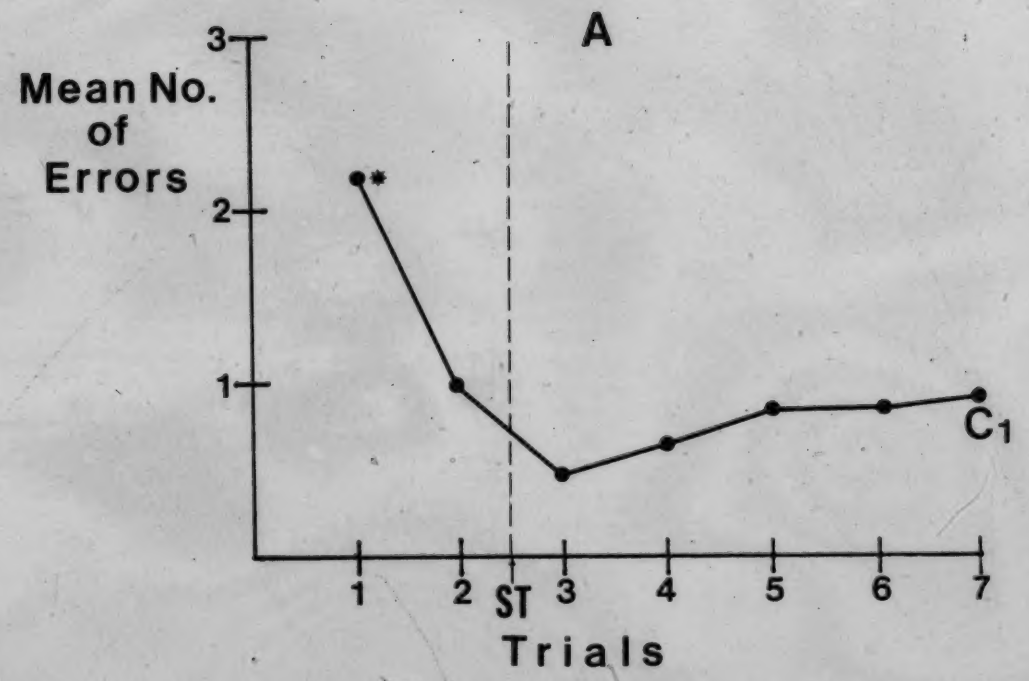


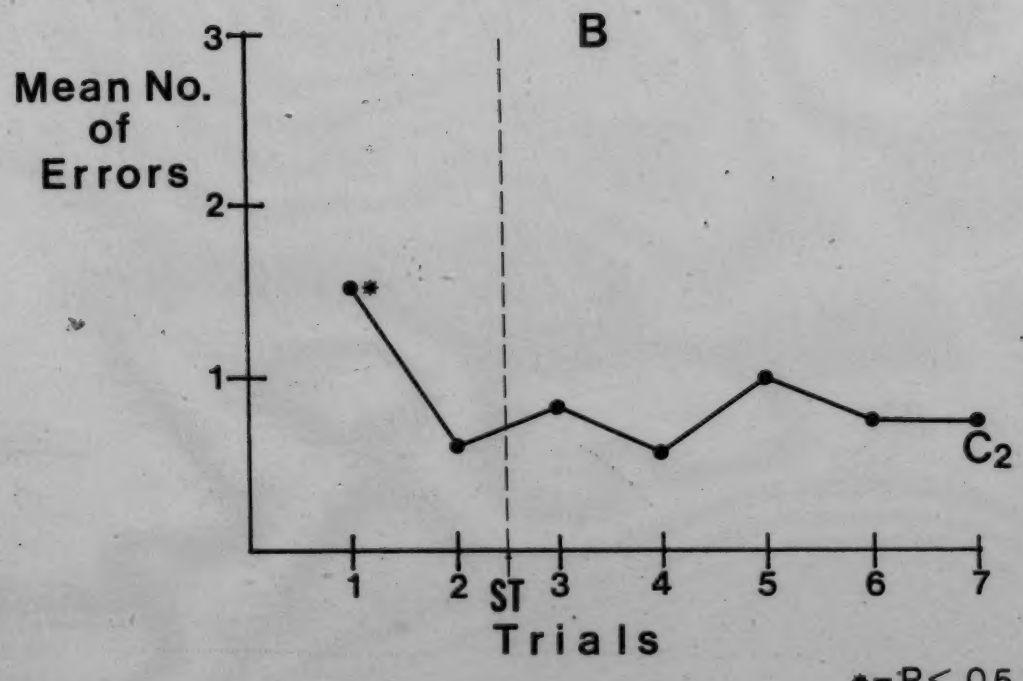
*- $\underline{P} < .01$

Figure 23. Graphs Showing Mean Error Performance For Groups C-1 And C-2 On Stimulation Days.

A. Graph shows results for group C-1. Note the decrease following trial one and the absence of change following stimulation.

B. Graph shows the results for group C-2. Note the same features as above.
The stimulation appears after trial two in these graphs since only long trials are included in the analysis.


A
Mean No. of Errors
3
2
1
C1
1
2
ST
3
4
5
6
7
Trials


B
Mean No. of Errors
3
2
1
C2
1
2
ST
3
4
5
6
7
Trials
*-P<.05

similar length trials were included in the analyses. Thus the three acquisition days consisted of two days in which the first trial was short, and one day where it was long. Thus the analyses compared the scores on the first trials for two acquisition days with the remaining short trials on those days. The analyses included a planned orthogonal comparison to discover differences between the first trial and the remaining trials. Table 7 provides a summary of the F-values obtained in each analysis and planned comparison, for each set of trials analysed. It can be seen that in every case a significant main effect for trials and a significant planned comparison was obtained. Figure 22 shows graphs of the mean errors made by all cue subjects for all acquisition days. (Remember that two analyses were run since one of these days began with a long trial.) Figure 23 provides graphs of the mean errors produced for groups C-1 and C-2 stimulation days. Note the consistently lower errors following trial one in both figures. The larger number of trials plotted in Figure 23 reflects the fact that stimulation trials were mostly long ones. Note also that following stimulation the error scores do not change significantly, a finding which agrees with the results for the distance analyses presented earlier (see Figure 18). This strongly suggests that the subjects were in fact learning the identity of the relevant cue on the first trial and displaying a subsequent win-stay strategy.

Further it suggests that the stimulation trials produced no impairment in the ability of the cue subjects to continue to employ a win-stay cue strategy involving a working memory component.

Discussion

It is interesting to note that the pattern of rearing during initial acquisition trials was quite similar in Experiments One and Two. In both cases the MAP subjects reared consistently more once the platform had been reached. This finding supports the conclusion proposed earlier that the CUE subjects quickly realize that the suspended cue signals the platform, hence they do not require an extensive knowledge of the external fixed environment.

Before discussing the present results of the stimulation trials, perhaps a brief review of the predictions underlying the design would be useful. The design included a working memory component common to both major experimental groups, namely the subject was required to remember, during the seven remaining daily trials, which was the relevant cue or platform location, once it had been discovered. According to the working memory hypothesis all stimulated subjects, regardless of the strategy they were required to use, should be impaired by electrical stimulation of the hippocampus. The spatial map hypothesis, however, predicts that only those subjects which employ a

spatial strategy to solve the task will be disrupted by the stimulation, thus in this case only the MAP subgroups would be expected to show impairment following stimulation. As the results of this experiment clearly illustrate, the spatial map hypothesis prediction is confirmed. Stimulation reliably produced severe disruption of MAP subjects ability to locate the platform, while the perfomance of the CUE subjects following stimulation appeared unaffected.

In order to state conclusively that these results simultaneously strengthen the spatial map hypothesis and weaken the working memory hypothesis it is necessary to demonstrate that the MAP subjects were relying solely on spatial strategies to perform the Morris water task. Confirmation that this is the case comes from two independent sources.

First the probe trials with the cheesecloth tent in Experiment One confirmed that the MAP subjects required the presence of fixed external cues to locate the platform. As far as possible the procedures used in dealing with the MAP subjects in Experiment Two were identical to those in Experiment One so that it is more than likely that the MAP subjects were forced to rely on a similar spatial strategy. The fact that the suspended cues were randomly located on each trial further insured that MAP subjects could not use these cues to locate the platform.

Second, the fact that identical results were obtained

for the stimulated MAP subjects on each bilateral stimulation day, regardless of whether the suspended cues were present during these trials, strongly suggests that the MAP subjects were employing spatial strategies throughout these trials.

It is important to comment on one aspect of the procedure used to train the MAP subjects. In retrospect, it is perhaps unfortunate that the platform locations for the MAP subjects were identical to the locations of the suspended cues for the CUE subjects. This lead to a situation in which a MAP subject which had learned that the platform could be in one of four locations on the first trial of any day, could locate the platform by simply swimming under each of the suspended cues in succession. Thus it became difficult to tell whether this searching behavior reflected a tendency on the part of the MAP subject to simply follow the cues or to actually visit the locations in which it previously found a platform. As the trials without suspended cues later showed, the cues were not necessary for the MAP subjects to find the platform, suggesting that they had, in fact, learned the platform locations. However, it is clear that the simple change of spatially separating the platform locations for the MAP group and the cue and platform combinations for the CUE group may have been a desirable modification.

Of perhaps even greater importance is the further

problem that such a design permitted the possibility that the CUE subjects could employ a simple orientation hypothesis to perform the task. From the perspective of the CUE subjects alone, clearly a better approach would have been to make the pattern of suspended cues totally random, with no fixed number of potential locations. However such an approach would not have allowed comparison of MAP and CUE subjects performing under identical stimulus conditions, since the working memory aspect for the MAP subjects required four fixed potential locations from which to choose. In this regard, the last section of the results section strongly suggests that the CUE subjects were in fact employing a win-stay strategy. The consistent decrease in errors following the first daily trial, and the large percentage of trials where the observed error score was lower than the expected both provide evidence in support of this conclusion.

Returning to the MAP subject results, a point of further interest relates to the symmetrical nature of the suspended cues and potential platform locations. This is the question of why the stimulation-induced disruption of groups M1 and M2 was substantially smaller in Experiment Two than in Experiment One. The peak mean value reached in Experiment One (see Figure 11) was well over 1000, while the peak mean value for Experiment Two was only 400, in spite of the fact that bilateral stimulation was used in

Experiment Two.

The explanation for this finding is to be found in the behavior of the stimulated MAP subjects in Experiment Two. It was noted that when introduced into the water all subjects had a distinct preference as to the direction in which they turned to begin swimming away from the wall. In non-stimulated subjects this turning behavior made little difference to the speed or distance covered in reaching the platform. Thus if a subject's preference was to turn right, and it was placed in the tank with the platform close on the left when the subject was facing the wall, it would simply turn right and then immediately head for the platform. The behavior of the stimulated MAP subjects usually contrasted sharply with this, and it is here that the difference with the MAP subjects in Experiment One may also be found. In this case bilaterally stimulated subjects would simply visit each potential location in sequence, starting with that closest to its position following its turn to face the center of the tank. This tendency to visit the locations in sequence usually led to the subject finding the platform rather quickly, although by a circular route. Thus the scores of stimulated MAP subjects, although they were significantly higher than the control subjects, were still much lower than the scores in the previous experiments. It is important to point out that there were a few stimulation trials in Experiment Two where the MAP

subjects would swim around randomly in the tank, as was the case in the previous experiments, but these were in a distinct minority.

The discovery of the significance of the turning preference led to a simple change in procedure to reveal the effect of the stimulation on these subjects. Following stimulation subjects would be placed in the tank at either of the two distant entry points, and the direction they turned was noted. The remaining trials alternated between these distant entry points until the final trial. At this point the subject was placed at an entry point close to the platform but with the platform on the side opposite to the subjects preferred turning direction. The subject would then invariably turn away from the platform and swim to each potential location until it reached the platform. Thus the final trial always consisted of a long circular pathway for the stimulated subjects during which they passed under several cues before reaching the platform. In contrast, the control subjects, also placed in the tank at an entry point which was close but on the opposite side simply continued their turn until lined up with the platform and swam directly to it, without passing under any cues. This is the reason for the striking difference in cues passed under seen in Figure 19, particularly on trial eight.

This pattern of swimming by the stimulated MAP subjects suggests some interesting possibilities with

respect to the effect of the stimulation. First it is of importance to note that the bilateral effect was much longer lasting than the unilateral effect. Second the circular path shown suggests that these subjects had to resort to a different strategy to solve the task, since the spatial strategy was disrupted. There is a distinct possibility that subjects had learned a simpler non-spatial strategy, one made possible by the conditions present in the tank. Specifically subjects could have employed the strategy of swimming around the circumference of the tank while remaining six to ten inches from the wall, since the subject's previous experience has been that the platform is invariably located at this distance from the wall. The circular swim path in the preferred direction supports this conjecture, since the strategy requires a regular path for efficient operation.

Some independent support for this conclusion is also available. First it is significant that the same circular swim paths were seen when stimulated MAP subjects were given trials with no suspended cues present. This suggests that the suspended cues themselves were not responsible for the circular swim paths. Second O'Keefe and Nadel (1978) suggest that subjects deprived of the hippocampal mapping system can still use orientation strategies in which the subject maintains the position of its body with respect to a given landmark or target. In this case the target would

be the tank wall and the strategy would consist of maintaining the set distance from the wall of the tank. Thus O'Keefe and Nadel (1978) would have no problem with this postulation that the MAP subjects were employing an alternate strategy when deprived of the spatial strategy by stimulation. Finally Sutherland (1983) has reported that hippocampally lesioned rats appear incapable of learning to use a spatial strategy to solve the Morris water task but often do appear to develope precisely the same orientation strategy as has been suggested above.

Problems and Ambiguities. Although the results of Experiment Two appear to be relatively straightforward, there are some possible objections which should be discussed. Primarily these relate to the fact that the experimental design and procedures employed were quite complicated, and as such, contained some potential sources of ambiguity concerning the results and their interpretation. It is this fact, coupled with the existence of a relatively old body of literature concerning the discriminative capacities of rats, which necessitates this section of the discussion.

A substantial amount of research on the capacity of the rat to discriminate stimuli and subsequently show good one-trial reversal has been published. Studies have shown that rats can learn to discriminate between two stimuli which differ on at least one dimension, such as brightness

(Lashley, 1930; Lashley, 1938; Bitterman & McConnell, 1954; MacCaslin, 1954; Gonzalez and Shepp, 1961), size and shape (Wodinsky, Varley, & Bitterman, 1954; Bitterman, Tyler, & Elam, 1955), and pattern orientation (Bitterman & McConnell, 1954; Sutherland, 1961). However there appear to be very few studies which report on the ability of the rat to make a simultaneous discrimination amongst four distinct stimuli. Indeed, those studies reporting acquisition of discrimination on the basis of visual cues generally involve long training periods for only two stimulus discriminations. Furthermore, when the experiments involved subsequent reversal procedures the performance level usually declined initially and, even at asymptote, still showed definite errors in successive reversals, especially so in one trial reversal paradigms. Thus, since this literature reports a limited capacity of rats to learn successive discrimination reversal with only two stimuli, it follows that even more errors, or perhaps even complete failure to acquire the task, might result from an experiment requiring simultaneous discrimination of four visual objects and subsequent one-trial reversal.

In defence of the present results two points may be made. First the majority of the early studies employed non-correction procedures in which the subject was not allowed to experience a reward if it made an initial wrong choice. In contrast, under the present conditions, each trial

could, in fact, be regarded as a series of up to four shorter trials, each involving the use of correction procedures. If the rat makes an error on its first choice in the water maze, it is then immediately allowed to correct itself and try again until it locates the platform. Each subject could then be considered to have received a substantially greater number of training trials involving correction procedures (i.e., the number of acquisition trials given times the total mistakes made during those trials). Hence the validity of directly comparing the present results with the earlier studies mentioned above may be called into question. Second the reader is reminded that the majority of the early studies cited above involved stimuli which differed in only one dimension. In contrast the stimuli used in the present study were chosen specifically to be highly distinctive and distinguishable. Thus the experiment contained a procedure which addresses this issue. Although the two points raised above are important, there remain additional issues to be raised.

The major problem with the experimental design has already been mentioned, namely, the fact that the stimulus objects were symmetrically placed in the pool, allowing the subjects to locate the platform quite quickly by simply visiting each suspended object in turn. This arrangement does tend to introduce an element of ambiguity into the experiment. This ambiguity derives from the fact that

subsequent scoring of errors for each CUE subject involved an certain degree of arbitrariness, as did the establishment of initial criteria for assigning an error. On the one hand, it is true that the analysis performed was reliable, given the chosen criteria, and that the results were statistically significant. On the other hand, if the criteria for scoring an error were to be relaxed slightly, it is highly likely that the tendency of the rats to swim a curved swim path would result in much larger error scores. This, in fact, is a major source of the ambiguity which is present in the data.

In addition, the camera used to record the trials was placed at a slight angle above the pool, in order to encompass the whole pool in the picture. This also led to problems in assessing errors, since it was often difficult to tell whether the subject had passed directly under the suspended cue in view of the camera angle involved. Thus although the results of the error analysis appear to indicate a high degree of learning on the part of the CUE subjects, the original means of obtaining the error scores require that these results be viewed with caution.

Some alternative explanations also bear mentioning. If one takes the approach that the error scores were in fact valid, and that the subjects were learning something, then the possibility arises that the subjects were employing incidental cues to locate the platform, rather than

actually discriminating amongst the suspended objects on each trial.

One possibility which arises is that the movements of the experimenter during the trial might provide a signal to the subject where the platform was. That is, after placing the subject in the pool, perhaps the experimenter was moving to the vicinity of the platform too quickly, in an attempt to remove the subject soon after it reached the platform. To investigate this possibility further the tapes were reviewed once again and scored according to the movements of the experimenter outside the tank. Each trial was placed in one of three categories; (1) no movement until the subject reached the platform, (2) clear movement in the direction of the platform before the subject reached it, and (3) impossible to ascertain the movement on the videotape for that trial. This latter category was only rarely used. In the vast majority of trials it appeared that this alternative was not viable. It was clear that the usual pattern folowed by the experimenter was to release the rat and then step back from the tank and remain still until at least the rat was headed for, or was in the immediate vicinity of, the platform. Thus on only approximately 10 % of the trials was there clear movement before the subject had reached the platform. However this is not sufficient to discount this alternative entirely, for the possibility still remains that the subjects could

have been perceiving some subtle signal given by the experimenter, which the camera could not pick up. This possibility must be allowed since in most cases the assessment of movement was made on the basis of only the experimenter's legs being visible in the videotape. This then is another source of ambiguity which must be considered in evaluating these results.

Another problem became apparent through repeated analysis of the videotapes. One unfortunate result of the randomization procedures concerning the entry point and platform location, was that the vast majority of trials were ones in which the expected error scores mentioned above were either zero or one. That is the platform was in either the first or second location in the preferred direction of turn on these trials. As has been pointed out before, it is impossible to distinguish between use of an orientation hypothesis and true discrimination learning as far as these trials are concerned, hence the number of trials which can provide such information is drastically reduced as a result of these randomization procedures. Relevant to this point a further assessment of the percent of trials on which the observed error scores was less than the expected error score was performed. In this case, only those trials on which the expected error score was two or more were considered. The results were interesting, although not conclusive. Using this approach, the values

for the number of trials on which the observed error rate was less than the expected error rate declined to between 25 % to 40%, substantially less than the high percentages obtained with all trials considered. Thus on those trials on which the orientation and cue hypotheses could be readily distinguished, there were still a relatively large number of instances where the observed error score was lower than the expected. It should also be pointed out that on the initial assessment including all the trials the high percentages obtained reflect many trials on which the expected error score is one and the observed is zero. Thus these trials may also be regarded as ones on which orientation and cue hypotheses may be distinguished. Thus, although the evidence is not conclusive, these analyses provide some good support for suggesting that the CUE subjects did learn the task as intended. It is unfortunate, though, that the randomization procedures employed, resulted in a reduced number of useful trials in the overall assessment of the performance of the rats. In future experiments care should be taken to avoid this problem.

One other problem concerns the lack of videotapes of early acquisition trials when shaping was occurring. These would have been most helpful in assessing the type of strategies developed by the subjects while the problem consisted of only two or three suspended objects. As it is

one can only speculate as to what they may have revealed.

Finally the tapes were assessed one last time to see if any evidence could be obtained to suggest that, on the first daily trial, the CUE subjects typically approached the suspended object which had been relevant on the previous day. Such a finding would offer support for the conclusion that the CUE subjects had learned the nature of the relevant object on the previous day. Here the evidence is quite clear cut, in that there was no discernable tendency in this direction. On the first trial subjects were much more likely to simply visit objects in sequence, than they were to make a special approach to the previously relevant object. Unless one assumes that the CUE subjects had, in fact, learned to reset their working memory over each day, this finding weakens the conclusion that the CUE subjects had learned to discriminate the relevant object on each new day.

Thus it can be seen that there are several problems which prevent the unquestioning acceptance of the conclusions drawn from this study. It is clear that before unequivocal conclusions may be drawn, further research which eliminates these sources of ambiguity must be performed. At the very least the type of follow-up study required must eliminate the symmetrical arrangement of the suspended objects, and involve a non-arbitrary means of scoring errors during a trial. Also the problem involving

randomization procedures needs to be dealt with. Finally it appears to be of great importance to ensure that clear videotapes of all trials are available for subsequent analysis.

In conclusion, it appears that although these sources of ambiguity can not be denied, the remaining results of the various analyses done, still point toward the conclusion that the CUE subjects did, in fact, learn the task assigned to them. While the presence of ambiguity must forestall any attempt to make firm conclusions to this effect, the weight of the findings provides strong encouragement to perform follow-up studies which are not as subject to ambiguity, in order to verify the conclusions which the present study invites.

General Discussion

Taking the results from both experiments as a whole, it is clear that the spatial map hypothesis of O'Keefe and Nadel (1978) is strongly and consistently supported. Experiments One and Two showed that animals forced to employ a spatial strategy to solve the task are severely impaired by brief low level unilateral electrical stimulation of the hippocampus. Further the impairment is transient, allowing recovery effects to be demonstrated within the series of daily trials given. In contrast, the animals forced to use a guidance strategy consisting of

approaching a single cue did not show any disruption in their ability to reach the platform efficiently. Such results are in agreement with the O'Keefe and Nadel (1978) position, since O'Keefe and Nadel (1978) postulate that subjects bereft of a functioning hippocampus should still be able to employ a simple guidance strategy. The importance of the present results is that they demonstrate disruption of a highly learned spatial task through stimulation techniques. In addition they show that stimulation immediately prior to performance produces a severe performance deficit. Previous to these findings the vast majority of studies involving stimulation to study spatial functions of the hippocampus have employed a consolidation paradigm in which stimulation was given at varying intervals after training and testing was not carried out until hours or even days had passed (Kesner, 1980). Thus very little research has been reported on the effects of disruption of hippocampal neural activity immediately before performance of the task in question was tested. This point will be returned to later in the discussion.

There is one study by Olton (1978) which is relevant to this part of the discussion. Olton trained rats to criterion in the eight-arm radial maze and then gave them four choices after which time they were confined in the center of the maze for about five minutes, during which time

some rats received hippocampal stimulation sufficiently strong to produce seizure afterdischarges. When the five minute period had elapsed, the subject was released and allowed to make further choices. The results were that only on stimulated trials did subjects enter any of the arms previously chosen on the initial four choices. This study is interesting in that it represents an intermediate position between a consolidation paradigm and a true performance paradigm. On the one hand the subjects would certainly have still been under the effects of the seizure level stimulation, but on the other hand the stimulation was given soon after the information which was crucial to the future performance of the rat had been obtained, ie. the arms chosen during the first four choices. Thus it qualifies as a consolidation study as well. Unfortunately it is consequently impossible to determine which aspect of the design was primarily responsible for the results. Also it must be remembered that the stimulation used was quite intense, which raises the possibility that extrahippocampal structures were undoubtedly involved. In any case, the results may be regarded as evidence of a spatial deficit since rats apparently entered arms randomly following stimulation, although Olton's own interpretation involves the working memory hypothesis.

It should be mentioned that, in a sense, the present studies may be viewed as involving consolidation paradigms.

Subjects receive a single learning trial each day in order to discover where the platform is located for that day. Since stimulation is given two or three minutes following this trial the claim may be made that the experiment used a consolidation paradigm. Consequently the information in the working memory store may not have been consolidated, resulting in disruption of subsequent performance in the MWT. There are two main points to be made at this point. First the time span of two to three minutes is generally considered to be long enough for consolidation to occur unimpaired. Second even if the obtained results are due to interference with ongoing consolidation processes, the effect has been shown to occur only in subjects using place strategies. As such the results still do not provide any support for Olton's hypothesis. since working memory is not restricted to spatial information, Olton and his colleagues would still predict impairment in subjects using non-spatial strategies.

While the results from both the present experiments support the spatial map hypothesis, those of Experiment Two specifically undermine the working memory hypothesis of Olton and his colleagues. The working memory hypothesis clearly predicts that hippocampal disruption should produce impairments in tasks involving working memory components. The present findings that stimulation did not affect groups C1 and C2 directly contradicts this prediction. This

conclusion is strengthened by the fact that the task on which the dissociation of the effect of hippocampal stimulation was obtained involved identical arrangements of physical stimuli. In other words, each group was exposed to the same configuration of stimuli, suspended and fixed alike. This represents a significant advantage over experiments which test predictions using different experimental setups. The direct nature of the comparison of the two hypothesis adds strength to the conclusion drawn.

In discussing the results of Experiment Two there appear to be two findings which need to be explained. First, as in Experiment One, what is the nature of the spatial impairment produced, and second why was there no effect seen in the stimulated CUE subjects?

The answer to the first question has already been extensively discussed. It is suggested that in this case virtually the same comments apply, since the training procedures were constant across the series of experiments. However there is one important difference which must be mentioned. In Experiment Two the MAP subjects learned that the platform could be in any of four locations on a given day. Each location was at a similar distance from the wall of the tank, and, perhaps even more important, all four locations were symmetrical. Finally the subjects were given over four times as much training prior to stimulation in Experiment Two. This combination of factors may have

encouraged the development of alternate strategies, which remained latent until the spatial strategy was eliminated as an alternative by stimulation. As suggested in the discussion of Experiment Two, this would account for the different nature of the behavior shown by MAP subjects in the two experiments.

While the failure of stimulation to have an effect on the CUE subjects does not contradict the spatial map hypothesis, it does present a considerable problem for the working memory hypothesis. It will be recalled that the direct origin of the present series of experiments was the radial maze study of Collier et al (1982). They interpreted the finding, that hippocampally stimulated rats often entered arms previously entered on that trial, as evidence of an impaired working memory. Similarly they viewed instances of stimulated rats entering the wrong arm for that day as evidence of a different type, or element, of working memory. Based on this interpretation they proceeded to regard the experiment as strong evidence in support of the working memory hypothesis. In the introduction to Experiment Two it was suggested that the findings could be more parsimoniously interpreted as a simple manifestation of a spatial impairment, which resulted in random entries of stimulated rats into the maze arms. The task was undeniably spatial, since the maze was in a fixed position and no distinctive intramaze cues were present. In light of

the present results it seems warranted to continue to regard the Collier et al results as evidence of a stimulation induced impairment in performance of a spatial task. It is important to point out that it is only the interpretation of the Collier study which is questioned. The data obtained, on the other hand, is replicated by Experiment Two and supports the conclusion that the working memory hypothesis is not confirmed in either study.

The important question remaining, then, is what is to become of the working memory hypothesis? As mentioned in the introduction to Experiment Two one severe problem which assails the working memory hypothesis is the lack of evidence based on non-spatial tasks. A second equally important problem facing the theory is that evidence is contradictory concerning the fact that significant reference memory errors often seem to accompany working memory errors, particularly during the initial testing trials. Such results pose problems for the working memory hypothesis. These two areas of concern will form the basis for the remaining discussion of the working memory hypothesis.

Although there appear to be two distinct problems for the working memory hypothesis, it must be pointed out that it is the presence of equal reference and working memory errors following hippocampal disruption which allow a spatial interpretation to be proposed. As has been

emphasized above, several studies involving spatial tasks have been cited in support of the working memory hypothesis (Olton & Papas, 1979; Collier et al, 1982). However the pattern of results typically reported in these studies often includes findings that lesioned subjects make as many reference errors, that is, entering non-baited arms, as working memory errors, that is, reentering baited arms on the same trial. This apparent equality of reference and working memory errors paints a compelling picture of the disrupted subject being unable to distinguish between the arms, all of which appear similar. Consequently the subject may recall that it has entered arms on that trial, but it may not be able to distinguish them spatially. Thus it is equally as likely to enter a non-baited arm as it is to reenter a baited arm. In fact, the spatial map hypothesis would predict that reference and working memory errors would be equal where a spatial task is involved.

The task employed in the Olton and Papas study (1979) has been rigorously analysed by its authors into its reference and working memory components. Since the hypothesis predicts only working memory errors following hippocampal disruption, the finding of any reference memory errors presents a problem. Olton and his colleagues point out that the reference memory errors decline after forty or so trials. However forty trials is not particularly quick in the context of the task employed and the time span

allows ample opportunity for changes due to some underlying process to become manifest. In this case it is possible a relearning phenomenon may account for the reference memory improvement. That is, after forty test trials, during which time the same conditions as those present during acquisition remained in effect, subjects may have been able to relearn to identify baited and non-baited arms.

In this regard it would certainly be interesting to compare the results obtained from earlier test trials for the mixed arm versus the adjacent arm condition run by Olton and Papas in their experiment, but such results are not adequately reported to allow this. This brings up the question of which results Olton and Papas felt were most valuable to concentrate on and subsequently publish. They limited their presentation of results to those originating from the final ten trials of postoperative testing. The total number of postoperative trials that they gave is 50 at a rate of one per day for five days a week. Olton and Papas gave two reasons for concentrating on the final segment of postoperative testing:

> " First it gives the animal with a damaged brain every opportunity to develop the appropriate behavior so that any effects due to changes in motivation, emotionality, or other irrelevant behavioral variables should be minimized. Second, permanent or long-term behavioral changes following brain damage are of more significance in making inferences about functional localization than transitory ones."
>
> (p. 670)

With respect to the second reason offered, it is entirely possible that, as suggested above, given a recovery period of 10 weeks, during which time testing was continually performed, the process of relearning may have occurred. The fact that the testing period was longer than the initial acquisition period supports this conjecture. Thus the terminal performance emphasized by Olton and Papas may actually have reflected the effects of the lesions combined with a relearning component. Presentation of the initial and middle testing results for the adjacent and mixed arm conditions would cast some light on this possibility. Such data is particularly important from the point of view of an approach which proposes that the data from the Olton and Papas study could be a demonstration of an impaired ability to perform spatial tasks.

This alternative interpretation would predict that the enduring working memory deficit would be more severe, and the reference memory recovery would be more dramatic in the adjacent arm condition. The logic underlying this prediction is as follows. The mixed arm condition requires the ability to make fine spatial discriminations to distinguish between arms, regardless of whether they are baited or not. Thus a spatial hypothesis would predict poor reference memory error recovery in the mixed condition. In contrast, the adjacent arm condition requires only a broad discrimation based on a large block of arms to distinguish

baited from non-baited arms, hence the spatial hypothesis would predict better recovery from reference memory errors in this condition. Further the adjacent condition would also make it more difficult to avoid working memory errors since subjects must make fine discriminations among similar arms in spatially similar locations. Consequently the spatial map hypothesis would predict that faster recovery from reference memory errors and a more enduring working memory deficit would result while the adjacent condition was in effect. Unfortunately the data required to test these predictions were not presented. The only mention Olton and Papas made of the result of the analysis of the adjacent versus mixed condition was to conclude that they did not differ over the final ten trials.

The fact that recovery of reference memory errors took forty trials, in itself provides some suggestion that the spatial hypothesis is valid. Olton has reported elsewhere that reference and working memory errors were nearly equal for the first few postoperative testing trials (Olton et al, 1980). Regardless of their preference to deal with the final trials, something must account for the initial test trial results, since the working memory hypothesis cannot. It is proposed by the present author that the spatial map hypothesis is a valuable candidate in this regard.

In their discussion of the reference memory error recovery Olton and Papas suggest an explanation based on

the interference characteristics of the working and reference memory tasks. In their analysis they suggest that the amount of interference produced in the task is substantially greater for the working memory than the reference memory component. Their reasoning is as follows. The more baited arms which are successfully visited on any given trial, the greater the subsequent interference in the working memory component of the task. Thus the working memory component would be expected to be a greater source of interference. However what is not clear is why interference would not be equally great in the reference memory component since performance of the overall task requires an equivalent memory of which non-baited arms have been entered on a trial. One interesting question that this raises is whether to score reentries to non-baited arms as reference or working memory errors. Olton and Papas (1978) do not address this aspect of the experiment in their rather brief discussion of interference.

This approach is echoed by Jarrard in a later study on the effects of selective lesions of the hippocampus on spatial behavior (Jarrard, 1980). In his experiment Jarrard employed a design similar to Olton and Papas, except that he used an eight arm maze. His findings were similar to Olton and Papas in that following lesioning hippocampals tended to make almost equal numbers of reference and working memory errors. As testing progressed the reference

memory error rate showed substantial recovery. In his discussion of these results, Jarrard raises the following important question:

> " While the distinction between reference memory and working memory is useful in analyzing different components of the task, a reasonable question to ask is whether the different errors reflect different underlying processes or whether a single process is, in fact, involved. Since CH (complete hippocampus), fimbria and dorsal fornix animals were impaired in the present experiment on both reference and working memory components of the task, it seems most parsimonious to conclude that a similar process must be involved." (p.204)

Jarrard agrees that the data may be accounted for by analyzing the interference characteristics present in each component, and it is this approach which leads him to conclude that a single factor, namely susceptibility to interference, may account for the difference between reference and working memory error rates. Thus Jarrard questions the explanatory nature of the working memory hypothesis, since his conclusion is that a single process underlies the differing error rates.

It is important to point out that the same interpretation based on the spatial map hypothesis which was applied above to the Olton and Papas study may also be applied to the Jarrard experiment. Once again a fixed radial maze was used and individual arms did not contain salient intramaze cues, indicating that the nature of the task was predominantly spatial. The finding of equal reference and working memory errors immediately following

lesioning may be regarded as further support for this approach.

The present results are interesting to consider in this connection. The design of Experiment Two included working and reference memory components for spatial and non-spatial tasks. If the gradual dissociation between working and reference memory error rates was due to an increase in working memory interference, then the spatial and non-spatial groups should have been equally affected by the presence of interference in the working memory component, and a distinction should have emerged in the data. What this approach does not predict is the observed dissociation based on the spatial nature of the task.

Further support for the O'keefe and Nadel hypothesis has also been reported in studies on man and other primates. A promising line of research which attempts to produce and study primate models of the severe amnesia which results from medial temporal lobectomy has been reported by Mishkin and his associates. Mishkin reported that simultaneous lesions of the hippocampus and amygdala greatly exacerbated the relatively minor impairment in recognition memory which followed lesion of either structure alone (Mishkin, 1978). In a follow-up to this initial report (Parkinson and Mishkin, 1982) it was reported that memory for spatial locations of objects was selectively impaired by hippocampal ablation but not by

amygdala ablation. These results produced the conclusion that the hippocampus is integrally involved with the rapid memorization of the location of objects. Such a conclusion is echoed by earlier recent work with human subject who have undergone right temporal lobectomies. Smith and Milner (used a task involving incidental recall of randomly placed objects on a table, and of their spatial locations. They reported that right temporal lobectomy patients were consistently impaired in recalling the location of the objects when compared to normal controls and left temporal lobectomy subjects. In addition right temporal lobectomy has also been shown to impair maze learning in intentional recall conditions (Corkin, 1965; Milner, 1965). Thus in addition to a wealth of corroborative animal literature, the O'keefe and Nadel hypothesis also receives support from primate and human studies which suggest a selective spatial memory function of the hippocampus. Of further interest is the finding that there seems to be a hemispheric asymmetry in humans which agrees with the generally accepted conclusion that right hemisphere structures are concerned with more holistic and spatial types of information.

Conclusions

One of the prime motivations for carrying out the three experiments described in this report was to investigate a perceived gap in research which has been

concerned with studying the role of the hippocampus in behavior. As has been stated, the literature is extremely sparse concerning studies which have examined the effect of various types of hippocampal disruptions on performance. Since it is generally agreed that the prime impetus for the large increase in the volume of hippocampal research over the last two decades was the need to find animal analogues of the severe memory deficit found to follow surgically-induced hippocampal damage in humans, it is somewhat surprising that the animal literature has essentially neglected the effect of ongoing disruption of the hippocampus during performance of a learned task. It is probable that the main reason for this neglect has been that the deficit in hippocampal humans was initially regarded as an inability to learn, or at least to consolidate information in some neural storage location. However this view has often been challenged in the last few years by researchers who suggest that the human deficit may be due to faulty retrieval during performance (Warrington and Weiskrantz, 1975). Given this change in approach, it would appear necessary to begin to concentrate on possible performance effects of disruption of the hippocampus, in addition to the more traditional concern with acquisition and consolidation.

If one accepts this reasoning, then it follows that the current low level of performance oriented hippocampal

research is a severe limitation on the data base upon which hypotheses as to the function of the hippocampus are founded. In other words, it may be premature to make sweeping conclusions concerning the function of the hippocampus until the complete nature of the deficit resulting from disruption of the hippocampus is fully characterized, and the large gap concerning performance effects is substantially reduced.

Since both major hypotheses propose a memory-based function of the hippocampus, it follows that performance variables should be of great interest to them. In this regard the working memory hypothesis has the distinct advantage of being based on studies which employed preoperative acquisition training, in order to examine the effects of interference with normal hippocampal neural function on performance of learned tasks. Indeed, Olton's stated research strategy is to employ post-acquisition lesioning.

The spatial map hypothesis, however, may be regarded as being equally concerned with the role of the hippocampus in acquisition and performance alike. According to O'Keefe and Nadel (1978) the hippocampus is essential for not only the retrieval of information from the map when performance is required, but also with the initial construction of the map. Unfortunately there is a large discrepancy in the proportion of acquisition versus performance research upon

which O'Keefe and Nadel (1978) base their hypothesis. The vast majority of studies cited in support of the spatial map hypothesis have involved pre-acquisition lesioning, with the result that pure performance data has been largely ignored. The previously discussed distinction between 'spatial map' and 'memory' which appears in the quotation taken from the introduction to Olton et al (1979), clearly illustrates this lack of emphasis on the memory aspect of the spatial map hypothesis.

In view of the above, it is perhaps even more impressive that results obtained from the present performance studies should agree so closely with the predicted results based on the spatial map hypothesis. The present finding that performance of the Morris water task following stimulation is impaired when the task is exclusively spatial, requiring a place strategy , and unaffected when the task requires a guidance strategy, is precisely the pattern of results predicted by the spatial map hypothesis.

Given this strong confirmation, from both experiments, of the spatial map hypothesis, and the lack of support for the working memory hypothesis, it must be concluded that the present series of experiments clearly indicates a crucial role of the hippocampus in the ability to employ a place strategy. The results obtained are complementary to the other main body of results obtained from the use of the

Morris water task, namely, those employing preacquisition lesioning. Taken together the results from these studies and the current ones strongly suggest that at least one of the major functions in which the hippocampus plays a part is the ability to efficiently construct and employ a neural representation of a familiar environment.

Having drawn this conclusion, it is important to discuss one final issue which has often been raised concerning both major hypotheses discussed in this paper. One of the prime criticisms levelled at both theories is that they propose a unitary function for a brain structure which receives input from a large number of cortical and subcortical areas. In his reaction to the working memory hypothesis, Ellen (1979) makes the following crucial point:

> " It is clear that Olton is attempting to subsume all aspects of the hippocampal deficit under a single functional rubric, much like those that argue that hippocampal damage results in a failure to process spatial cues or to form a cognitive map. In either case it is postulated that the hippocampus has a unitary function and that the lesion impairs this function. It is remarkable that investigators still attempt to find unitary functions for structures as complex - with respect to input-output connections, neurotransmitter mechanisms, and so forth - as the hippocampus. Given the many sources of input to the hippocampus, and its extensive projections, unitary conceptualizations (sic) of hippocampal function would seem to be overly simple and unwarranted. Rather, a more reasonable strategy would seem to be the assumption that the hippocampus is involved in a number of behavioral functions and that different behavioral tasks may tap into these functions to varying degrees. It would then become important to determine for any given task whether, for example, spatial/non-spatial cues

> play a greater role in determining performance than the operation of a working memory rather than a reference memory system."
> (Ellen, p.329)

While Ellen makes this point rather forcefully it may be argued that the effort is not altogether necessary. It is probably the case that neither O'Keefe and Nadel, nor Olton and his colleagues seriously suggest that the hippocampus is solely involved in spatial mapping or working memory respectively. Most likely the reason this criticism has been voiced is that each author has been concerned with presenting the strongest possible case for his approach, one which does not admit many alternatives to the discussion. Hence in dealing with a single hypothesis it is inevitable that some may view the author as suggesting a unitary function. The problem is easily eliminated by slightly modifying the claims of those presenting the hypothesis. Instead of postulating that "The hippocampus mediates function A", all that is necessary is to restate the assertion, such that it becomes "One of the functions" or "The major function". In this way the hypothesis being forwarded loses little impact, yet the overall effect is to reduce the rigidity of the position espoused.

Some concrete evidence suggesting that Olton et al readily admit to alternative functions of the hippocampus is found in their discussion of the Becker et al study described in the introduction to Experiment Two. In

commenting upon the impairments found in this study they conclude:

> " The selective impairment of rats with fimbria-fornix lesions in the retention of the location discrimination but not in the retention of the object discrimination does not support the idea that only the memory requirement of a task is important in determining whether or not a deficit will occur following damage to the hippocampal system."
>
> (Becker et al, p.244)

Thus the Becker et al experiment is important in that it illustrates a situation where the results clearly show that the hippocampus is involved in functions other than mediating working memory. In the same vein the present results invite a similar conclusion.

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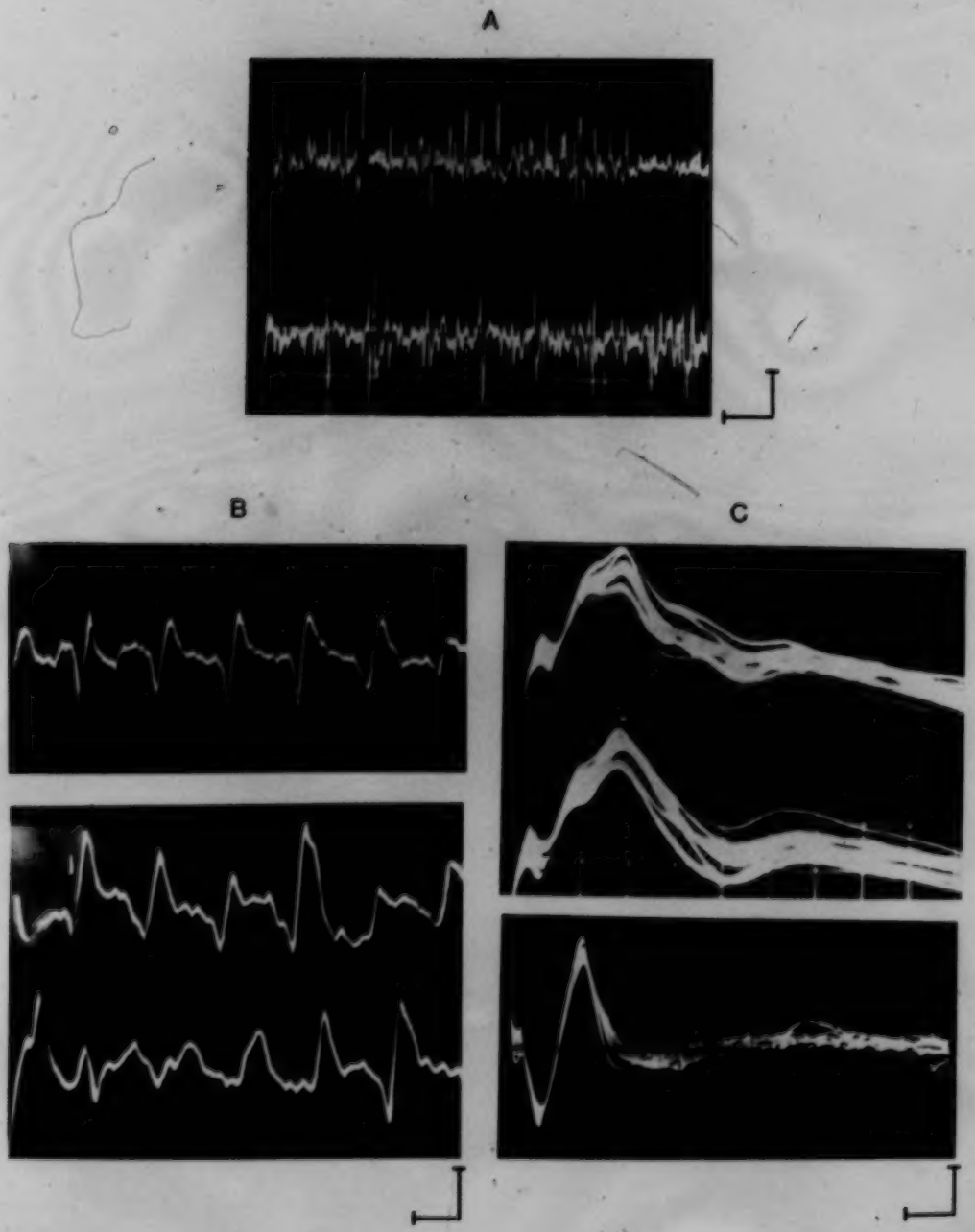
APPENDIX ONE

Plate 1. EEG Records Obtained During Surgery In Experiment One.

Part A shows the bursting patterns recorded as the electrode entered the pyramidal layer (top trace) and then the molecular layer of the dentate gyrus (bottom trace). Note that the pyramidal burst has more spikes over 100 microvolts than the dentate granule burst (17 vs 2). Also the frequency of the spikes is slightly greater in the pyramidal record. Calibration: 50 microvolts & 0.5 sec.

Part B shows representative samples of theta activity from the hippocampal region. The top trace is from the dentate gyrus, and the middle and bottom traces are from the dentate gyrus and ipsilateral pyramidal area of a different subject. Calibration: 200 microvolts & 0.5 sec.

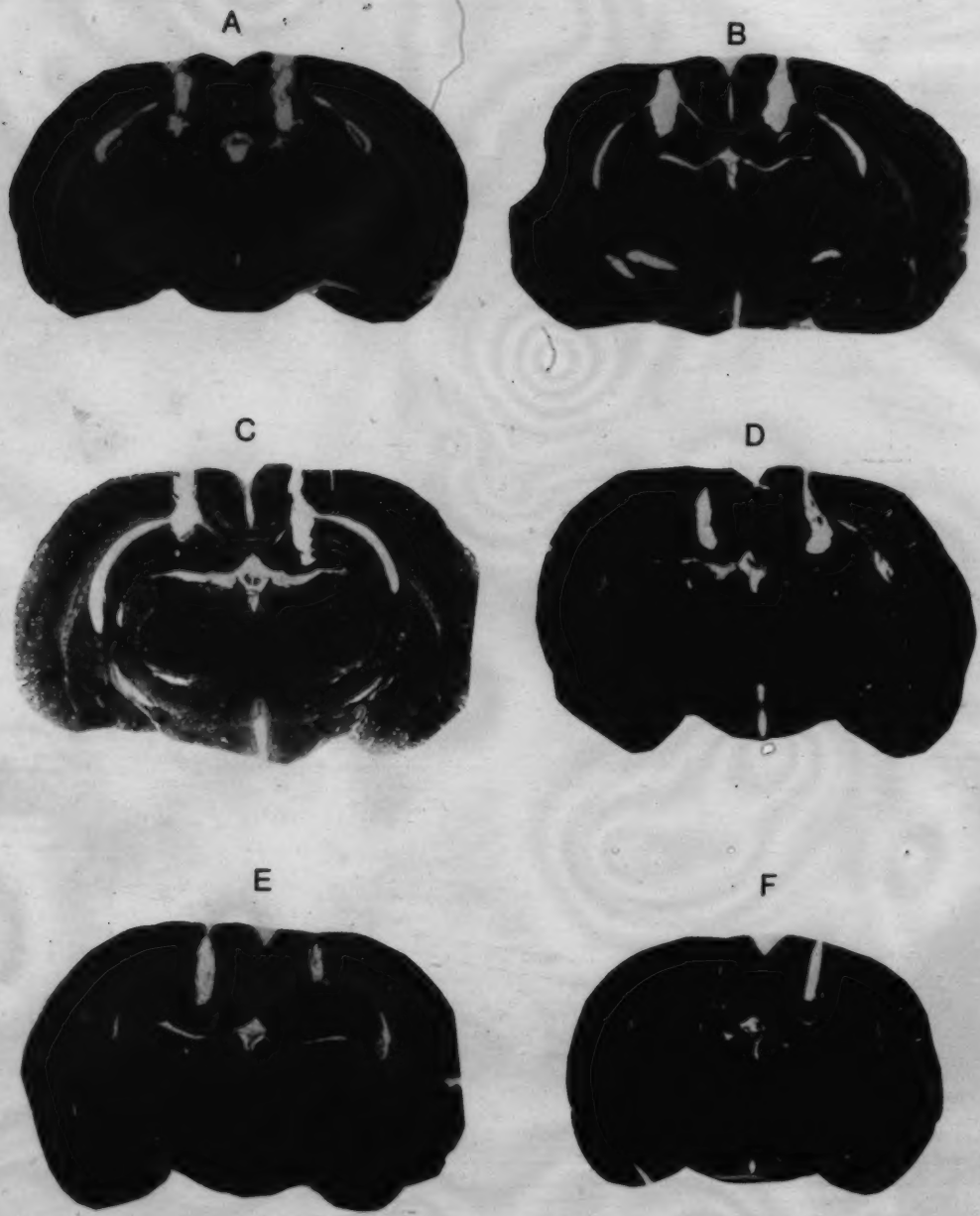
Part C shows sample evoked potentials obtained during surgery. The top and middle traces show similar evoked potentials obtained from the top and middle electrodes of one subject. The stimulating electrode situated in the contralateral dentate gyrus was stimulated at a low frequency (12 volts, 2 Hz, 0.2 ms pulse width) such that each record in Part C consists of multiple evoked potentials superimposed on each other.
The bottom trace shows an evoked potential obtained from the dentate gyrus contralateral to the stimulating electrode (stimulus parameters were 9 volts, 1 Hz, 0.2 ms pulse width).
Calibration: 200 microvolts & 10 msec.


A
B
C

APPENDIX TWO

Plate 2. Photomicrographs Showing Electrode Placements For Subjects In Experiments One and Two.

Sections A to D provide representative sections from Experiment One, showing the electrode track and the tip placement. Sections E and F are from subjects in Experiment Two. Note that the elctrode tracts are wider in the first four sections, and also note the presence what appears to be infected tissue. Sections E and F show examples of subjects classified as having large and medium infections respectively.


A
B
C
D
E
F